



**HEART FAILURE WITH PRESERVED EJECTION FRACTION: FROM
PROGNOSIS TO CARDIAC EFFECTS**

THE ROLE OF FITNESS, PHYSICAL ACTIVITY AND EXERCISE TRAINING

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“Coming together is a beginning; keeping together is progress; working together is success” (Henry Ford)

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RESUMO

Racional: A insuficiência cardíaca com fração de ejeção preservada (ICFEP) continua refratária às terapias disponíveis, sendo que as diretrizes atuais de tratamento destacando a importância de se concentrar na melhoria do bem-estar do paciente ou de outras componentes relacionadas à saúde. Há também uma crescente consciencialização sobre a necessidade de novas abordagens, não só para o tratamento da ICFEP, mas também para sua prevenção, através da identificação precoce e da gestão de fatores de risco modificáveis. A aptidão física ou atividade física (AF) vem sendo reconhecidas como importantes fatores de risco modificáveis para a prevenção da ICFEP e para a gestão dos sintomas cardinais, como a intolerância ao exercício e a qualidade de vida (QV). Do ponto de vista clínico, a aptidão física e a AF podem ser consideradas importantes alvos para otimizar os cuidados de saúde desses pacientes. No entanto, existem algumas lacunas neste campo que tornam desafiante o desenvolvimento de intervenções focadas na aptidão física e na AF. Primeiro, sendo a aptidão física um constructo multidimensional, é importante entender como a ICFEP afeta das diferentes componentes da aptidão física e qual delas é mais representativa das componentes relacionados à saúde. Segundo, para um melhor aconselhamento e prescrição de AF para estes doentes, é essencial que os instrumentos utilizados para medir os níveis de AF sejam precisos e confiáveis. Embora o uso de questionários possa ser de fácil aplicação na prática clínica, ainda não se sabe se a AF auto reportada se correlaciona com a AF objetivamente medida em pacientes com ICFEP. Terceiro, embora haja alguma evidência de que o exercício físico possa melhorar a função diastólica em pacientes com ICFEP, os mecanismos subjacentes a essas mudanças permanecem mal compreendidos.

Objetivo: No presente trabalho propomos: i) estudar a associação entre os diferentes componentes da aptidão física e as dimensões da QV em pacientes com ICFEP; ii) examinar quais das componentes da aptidão física estão independentemente relacionados a diferentes dimensões da QV; iii) determinar a validade do Questionário Internacional de AF (IPAQ) comparado com medidas objetivas derivadas da acelerometria em pacientes com ICFEP; iv) descrever os padrões diários de AF e tempo sedentário, e avaliar quais padrões estão melhor associados aos indicadores prognósticos; v) avaliar os efeitos do exercício físico na função e estrutura do ventrículo esquerdo (VE), e alterações moleculares subjacentes, utilizando um modelo animal de ICFEP com ratos obesos ZSF1.

Métodos: Para atingir os objetivos propostos, avaliamos 24 pacientes com ICFEP (Estudo I e II). Foi avaliada a aptidão física [equilíbrio dinâmico e mobilidade (*8-foot up and go*), força dos membros superiores (força de preensão manual), aptidão cardiorrespiratória (6 minutos de marcha), composição corporal (índice de massa corporal)] e a QV (*Minnesota Living With Heart Failure Questionnaire*). A atividade física foi avaliada pela versão curta do IPAQ e por acelerómetros (ActiGraph GTX3). No Estudo III, utilizamos o modelo animal de ratos obeso ZSF1. Os animais foram divididos aleatoriamente em um grupo de

exercício ou sedentário. No final do protocolo, todos os animais foram submetidos ao teste de tolerância ao esforço e à avaliação hemodinâmica invasiva. Após o sacrifício, amostras de sangue e do VE foram coletadas para análise.

Resultados: No Estudo I, nossos dados sugerem que o equilíbrio dinâmico e mobilidade é a componente da aptidão física que melhor se associa à QV em pacientes com IC-FEP. No Estudo II, nossos dados sugerem que a versão curta do IPAQ subestima o tempo sedentário e sobrestima a AF de intensidade moderada-vigorosa (AFMV). Além disso, diariamente os pacientes passam reduzido tempo em APMV, que foi a única categoria de intensidade de AF associada positivamente a indicadores prognósticos. Finalmente, no Estudo III, mostramos que exercício físico crônico melhorou a capacidade de exercício, atenuou a rigidez do VE e reduziu os níveis circulantes de citocinas inflamatórias e marcadores de disfunção endotelial e estresse oxidativo, em ratos com IC-FEP.

Conclusões: Os resultados sugerem que a aptidão física, particularmente o equilíbrio dinâmico e a mobilidade, deve ser um alvo da avaliação de pacientes com IC-FEP por se relacionar com a QV. Além disso, os dados da AF auto reportados podem ser inapropriados para o aconselhamento e prescrição adequada de alterações do estilo de vida. Em relação aos hábitos de AF, nossos dados revelam a importância de incentivar os doentes com IC-FEP a aumentarem os níveis de APMV. Finalmente, o exercício físico parece ter um impacto positivo na rigidez do VE através da modulação das propriedades intrínsecas dos cardiomiócitos e da remodelagem da matriz extracelular.

Palavras-chave: Aptidão física, atividade física, exercício físico, insuficiência cardíaca com fração de ejeção preservada, qualidade de vida, capacidade funcional, função diastólica.

ABSTRACT

Rational: Heart failure with preserved ejection fraction (HFpEF) continues to be refractory to available therapies, with current treatment guidelines highlighting the importance of focusing on the improvement of patient's well-being or other health-related outcomes. There is also an increasing awareness of the need for novel approaches not only for the treatment of HFpEF but also for its prevention through the early identification and management of potential modifiable contributing risk factors. Physical fitness or physical activity (PA) are becoming recognized as key modifiable factors for the prevention of HFpEF and management of cardinal symptoms such as exercise intolerance and quality of life (QoL). From a clinical perspective, physical fitness and PA may be considered important targets if we aim to maximize the health care of these patients. However, there are some gaps in this field that may challenge the effectiveness of physical fitness and PA based-interventions. First, because physical fitness is a multicomponent construct, it is important to understand how this syndrome affects the different components, and which of them is better representative of health-related outcomes. Second, in order to provide tailored counselling and prescription to HFpEF patients, it is crucial that the instruments that we use to measure PA levels are accurate and reliable. While the use of questionnaires may be easy to apply in the clinical practice, it remains to be confirmed if self-reported and objectively measured PA is correlated in HFpEF. Third, while there is some evidence that exercise training can improve diastolic function in HFpEF patients, the mechanisms underlying these changes remain poorly comprehend.

Purpose: In the current work, we propose to: i) study the association between different components of physical fitness and the dimensions of QoL in HFpEF patients; ii) examine which of the physical fitness components are independently related to different dimensions of QoL; iii) to determine the validity of the International Physical Activity Questionnaire (IPAQ) against objective measures from accelerometry in HFpEF patients; iv) to describe the patterns of daily PA and sedentary time and assess which is better associated with prognostic indicators; v) evaluate the effects of exercise training on LV function and structure, and underlying molecular changes, using the ZFF1 obese animal model of HFpEF.

Methods: In order to accomplish the proposed aims, we evaluated 24 HFpEF patients (Study I and II). Patients were assessed for physical fitness [dynamic balance and mobility (8-feet-up-and go test), upper body strength (handgrip strength), cardiorespiratory fitness (CRF) (6-minute-walking test), body composition (body mass index)] and for QoL (Minnesota Living With Heart Failure Questionnaire). Physical activity was assessed through the IPAQ short version and triaxial accelerometry (ActiGraph GTX3). In order to evaluate the effects of exercise training on LV function and structure, and underlying molecular changes (Study III), we used the ZSF1 obese animal model. Animals were randomly divided in a training or sedentary group. At the end of the protocol, all animals were submitted to exercise tolerance test, and invasive hemodynamic evaluation. After sacrifice, blood and left ventricular samples were collected for analysis.

Results: In Study I, our data suggests that dynamic balance and mobility is the only physical fitness component that better capture QoL in HFpEF patients. In Study II, our data suggests that the IPAQ short version underestimates sedentary time and over-estimates MVPA. In addition, patients spent only a minority of their time involved in moderate-to-vigorous PA, which was the only PA pattern positively associated with prognostic indicators. Finally, in Study III, we show that chronic exercise training improved exercise capacity, attenuated LV stiffness and reduced circulating levels of inflammatory cytokines and markers of endothelial dysfunction and oxidative stress in rats with HFpEF.

Conclusions: Our data suggests that physical fitness, particularly dynamic balance and mobility, should be evaluated in HFpEF patients, once that it is associated with QoL. Also, physical activity data gathered solely by self-reported instruments may lead biased counselling and prescription. Regarding to PA patterns, our data points for the importance of recommending HFpEF to more engaged in MVPA. Finally, exercise training seems to positively impact left ventricular stiffness by modulating both cardiomyocyte's intrinsic proprieties and extracellular matrix remodelling.

Keywords: Physical fitness, physical activity, exercise training, heart failure with preserved ejection fraction, quality of life, functional capacity, diastolic function.

LIST OF ABBREVIATIONS

6MWT	6-minute walk test
8FUG	8-foot up and go test
ACE-i/ARB	angiotensin-converting enzyme inhibitor and angiotensin receptor blocker
AF	atrial fibrillation
BMI	body mass index
BNP	b-type natriuretic peptide
CAM:	cell adhesion molecule
cGMP	cyclic guanosine monophosphate content
CO	cardiac output
COPD	chronic obstructive pulmonary disease
CPM	counts per minute
CRF	cardiorespiratory fitness
DBP	diastolic blood pressure
E/A	mitral ratio of peak early to late diastolic filing velocity
E/e'	ratio of early mitral transmitral flow velocity with early diastolic velocity of the mitral valve annulus
EF	ejection fraction
ESC	European Society of Cardiology
HF	heart failure
HFpEF	heart failure with preserved ejection fraction
HFrfEF	heart failure with reduced ejection fraction
HR	heart rate
IPAQ	International physical activity questionnaire
ICAM	intercellular adhesion molecule

LA	left atrial
LAVI	left atrial volume index
LPA	light physical activity
LV	left ventricle
LVEDP	left ventricular end diastolic pressure
LVEF	left ventricular ejection fraction
LVH	left ventricular hypertrophy
LVMi	left ventricular mass index
MET	metabolic equivalent
MLHFQ	Minnesota Living with Heart Failure Questionnaire
MMP	matrix metalloproteinase
MRA	mineralocorticoid receptor antagonist
MVPA	moderate to vigorous physical activity
NHYA	New York Heart Association
NO	nitric oxide
NT-proBNP	N-terminal pro B-type natriuretic peptide
PA	physical activity
PASP	pulmonary artery systolic pressure
PCWP	pulmonary capillary wedge pressure
PKA	protein kinase A
PKCα	protein kinase C α
PKG	protein kinase G
QoL	quality of life
RCT	randomized controlled trial
RER	respiratory gas exchange ratio
ROS	reactive oxygen species

RV	right ventricular
SBP	systolic blood pressure
sGC	soluble guanylate cyclase
SV	stroke volume
TGF-β	transforming growth factor β
TIMP	tissue inhibitors of metalloproteinases
TNF-α	tumour necrosis factor alfa
TR	tricuspid regurgitation
VCAM	vascular cell adhesion molecules
VE	minute ventilation
VE/VCO₂	ventilatory equivalents for carbon dioxide output
VE/VO₂:	ventilatory equivalents for oxygen
VO₂	oxygen consumption

CHAPTER I

GENERAL INTRODUCTION

1. OVERVIEW OF HEART FAILURE

Heart failure (HF) is a clinical syndrome caused by impaired cardiac structure and/or function, resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress, which leads to exercise intolerance (Ponikowski et al., 2016). It affects approximately 26 million people worldwide, and accounts for an estimated annual health care cost of \$31 billion (Mozaffarian et al., 2016). In Portugal, the EPICA study reported a prevalence of HF ranging from 7.6% in the 60–69-year-old group to 16.1% in patients >80 years (Ceia et al., 2002). In addition, it is estimated that the prevalence of HF in Portugal will increase by around 7% in 2018, 30% in 2035 and 33% in 2060 (Fonseca et al., 2018). Despite the evident progresses in the treatment of cardiovascular disease over the past decades, the incidence and prevalence of HF have not decreased, survival has slightly improved, and morbidity remains excessively high (Meta-analysis Global Group in Chronic Heart Failure, 2012). One of the challenges and barriers in successfully treating HF is the phenotypic heterogeneity found on this condition (e.g. different pathophysiology, disease severity and response to therapy) (Abbate et al., 2015).

Heart failure is usually associated with reduced left ventricular ejection fraction (LVEF) (HFrEF), but it is now recognized that HF also encompasses a wide range of patients with normal LVEF, defined as HF with preserved ejection fraction (HFpEF) (Ponikowski et al., 2016). Heart failure with preserved ejection fraction represents approximately 50% of patients with HF, and it is the main form of HF in adults aged above 65 years old (Dunlay et al., 2017). It is estimated that by 2020, the relative prevalence of HFpEF and HFrEF are predicted to reach 69% and 31%, respectively (Steinberg et al., 2012). Patients with HFpEF exhibit a long-term prognosis similar to HFrEF, with a five-year survival of less than 50% (Shah et al., 2017). In addition, while there are a few pharmacologic and non-pharmacological therapies demonstrated to improve survival and reduce HF hospitalization in HFrEF, the scenario is less satisfactory for patients with HFpEF as, up to date, no recognized therapies have shown important reductions in morbidity or mortality (Nanayakkara et al., 2018).

Given the increasing prevalence of HFpEF, there is an urgent need of clinical and translational research to better characterize this population and to identify effective strategies for the prevention and management of HFpEF.

2. HEART FAILURE WITH PRESERVED EJECTION FRACTION

2.1. Definition and diagnosis criterion

Heart failure with preserved ejection fraction is a clinical syndrome characterized by normal ejection fraction at the expense of increased left ventricular filling pressures (Andersson & Vasan, 2014).

The diagnosis of HFpEF remains defiant, once that signs and symptoms are often non-specific and do not help to distinguish between HF and other clinical conditions presenting similar manifestations such as in obese individuals, in the elderly and in patients with chronic lung disease (Ponikowski et al., 2016). Typical symptoms of HF are breathlessness, ankle swelling, fatigue and reduced exercise tolerance, which may be accompanied by signs such as elevated jugular venous pressure, pulmonary crackles and peripheral oedema (Ponikowski et al., 2016). Patients are usually classified into subgroups based on exercise limitation and their symptoms using the New York Heart Association (NYHA) functional class. Functional capacity is classified with objective assessment (class A to D), while patient's symptoms are based on how much they are limited during physical activity (class I to IV) (Dolgin, 1994).

According to current European Society of Cardiology (ESC) recommendations (Ponikowski et al., 2016), the diagnosis of HFpEF requires the following conditions to be fulfilled: i) signs and/or symptoms of HF (mentioned above); ii) preserved ejection fraction (defined as LVEF >50%); iii) elevated levels of natriuretic peptides (BNP >35 pg/mL and/or N-terminal pro B-type natriuretic peptide (NT-proBNP) >125 pg/mL); and objective evidence of other cardiac functional and structural alterations underlying HF. Finally, in case of uncertainty, a stress test or invasively measured elevated left ventricle (LV) filling pressure may be needed to confirm the diagnosis.

Key structural alterations include a left atrial volume index (LAVI) >34 mL/m² or a left ventricular mass index (LVMI) ≥115 g/m² for males and ≥95 g/m² for

females. Key functional alterations are an $E/e' \geq 13$ (ratio of early transmitral diastolic flow velocity to tissue Doppler early mitral annular diastolic velocity) and a mean e' septal and lateral wall <9 cm/s (Ponikowski et al., 2016). A summary of the diagnostic criteria for HFpEF according to ESC recommendations is presented in Table 1.

Table 1: Diagnostic criteria for HFpEF according to ESC guidelines.	
History and examination	Symptoms and signs of HF
Ejection fraction	≥ 50
Natriuretic peptides	BNP >35 pg/mL and/or NT-proBNP > 125 pg/mL
Imaging	Cardiac structural alterations LAVI >34 mL/m ² LVMI ≥ 115 g/m ² (males) ≥ 95 g/m ² (female) And/or cardiac functional alterations E/e' mean septal-lateral ≥ 13 Mean e' septal/lateral wall <9 cm/s Additional indirect measures Reduced global longitudinal strain Elevated PASP (from TR velocity)
ECG	AF, LVH, repolarization abnormalities
Exclusions	Exclude other known causes of HF
Further testing in case of uncertainty	Diastolic stress test E/e' , PASP, strain, SV and CO or invasive assessment of LV pressures Rest PCWP ≥ 15 mmHg (≥ 25 mmHg with exercise) or LVEDP ≥ 16 mmHg \pm change with exercise*

Adapted from (Zakeri & Cowie, 2018). AF: atrial fibrillation; BNP: b-type natriuretic peptide; CO: cardiac output; HF: heart failure; HFpEF: heart failure with preserved ejection fraction; LAVI: left atrial volume index; LV: left ventricular; LVEDP: left ventricular end diastolic pressure; LVH: left ventricular hypertrophy; LVMI: left ventricular mass index; NT-proBNP: N-terminal pro B-type natriuretic peptide; PASP: pulmonary artery systolic pressure; PCWP: pulmonary capillary wedge pressure; SV: stroke volume; TR: tricuspid regurgitation.

2.2. Epidemiology

The prevalence of HFpEF ranges from 1.1% to 5.5% and represent 40%-70% of HF cases (Oren & Goldberg, 2017). In Portugal, it is estimated that

126.898 patients will be suffering from HFpEF in 2018 (Fonseca et al., 2018). The prevalence of HFpEF is expected to increase mainly because of the rise in global life expectancy, significant advances in diagnosis, and high exposure to risk factors (Dunlay et al., 2017). Patients with HFpEF are more likely to be women and old aged, with a higher prevalence of cardiovascular comorbidities (e.g. hypertension, atrial fibrillation and valvular disease) (Dunlay et al., 2017) and non-cardiovascular comorbidities (e.g. anaemia, chronic kidney disease and cancer) (Lund et al., 2014).

Outcomes following hospitalization for decompensated HFpEF are quite poor, with a mortality rate of 5.9% at 30 days and 33% after 1 year (Ziaeeian et al., 2017). In-hospitalization mortality rate was estimated to range from 1.6% to 5.1% (Goyal et al., 2016). Regarding all-cause mortality, according to data from randomized controlled trials (RCT), it can range from 13% to 23% over a mean follow-up period of 26-50 months (Vaduganathan et al., 2016). The main causes of death in HFpEF patients often varies according to the study setting. Results from a meta-analysis suggest that cardiovascular causes are the predominant mode of death in HFpEF, especially from sudden death and worsening of HF (MAGGIC, 2012). However, as opposed to HFrEF, non-cardiovascular deaths also account for a significant proportion of deaths, with some data suggesting that they may even represent the most prevailing cause of mortality in HFpEF patients (Vaduganathan et al., 2017).

2.3. Risk Factors

The understanding of the contributing role of each risk factor implicated in the development of HFpEF is a hard task, once there is a wide inter-individual variation, and their possible interactions can also modulate the chances of developing HFpEF (Andersson & Vasan, 2014). Overall, the most important risk factors for the development of HFpEF include advanced age, female gender, and physical inactivity (Ponikowski et al., 2016). In addition, cardiovascular risk factors are found to be highly prevalent in HFpEF in population-based studies and registries, which include overweight/obesity (83%) (Haass et al., 2011), hypertension (60-80%) (Dunlay et al., 2017), coronary arterial disease (20-76%)

(Lam et al., 2011), diabetes mellitus (20-45%) (Dunlay et al., 2017), and atrial fibrillation (15–41%) (Fonarow et al., 2007; Olsson et al., 2006). In addition, the presence of non-cardiovascular comorbidities is highly prevalent in HFpEF patients, likely contributing to poor outcomes. Non-cardiovascular comorbidities include renal dysfunction, chronic obstructive pulmonary disease, anaemia, cancer, liver disease, peptic ulcer disease, among others (Ather et al., 2012).

2.4. Pathophysiology

As illustrated in Figure 1, HFpEF is caused by a complex interaction of multiple impairments in parallel with diastolic dysfunction and ventricular stiffness (Fontes-Carvalho & Leite-Moreira, 2011; Zakeri & Cowie, 2018). It is frequently associated with impairments in ventricular systolic reserve function, heart rate reserve and rhythm, atrial and renal dysfunction, vascular stiffness, endothelial dysfunction, impaired vasodilatation, pulmonary hypertension, and peripheral alterations, such as abnormalities of skeletal muscle (Borlaug, 2014; Fontes-Carvalho & Leite-Moreira, 2011; Oren & Goldberg, 2017; Zakeri & Cowie, 2018).

Patients with HFpEF are most often characterized at the cardiac level by a non-dilated LV, concentric LV hypertrophy or concentric LV remodelling, and diastolic dysfunction (Oren & Goldberg, 2017). The maladaptive ventricular hypertrophy of the LV is associated with an increase in myocardium stiffness due to the increased interstitial fibrosis (van Heerebeek et al., 2012) and changes in the expression and level of phosphorylation of cytoskeletal proteins (Castro-Ferreira et al., 2011; Hamdani et al., 2013). This leads to functional changes that manifest as incomplete myocardial relaxation and increased filling pressures of the LV (Chaturvedi et al., 2010; Ferreira-Martins & Leite-Moreira, 2010; van Heerebeek et al., 2012). In addition, other contributing factors were identified such as LV systolic dysfunction (Kraigher-Krainer et al., 2014), right ventricular dysfunction (Guazzi et al., 2011), chronotropic incompetence (Brubaker et al., 2006), autonomic deregulation (Borlaug et al., 2006), impaired vascular (Schwartzberg et al., 2012), pulmonary and renal functions (Maurer et al., 2007), and impaired skeletal muscle function (Haykowsky et al., 2013).

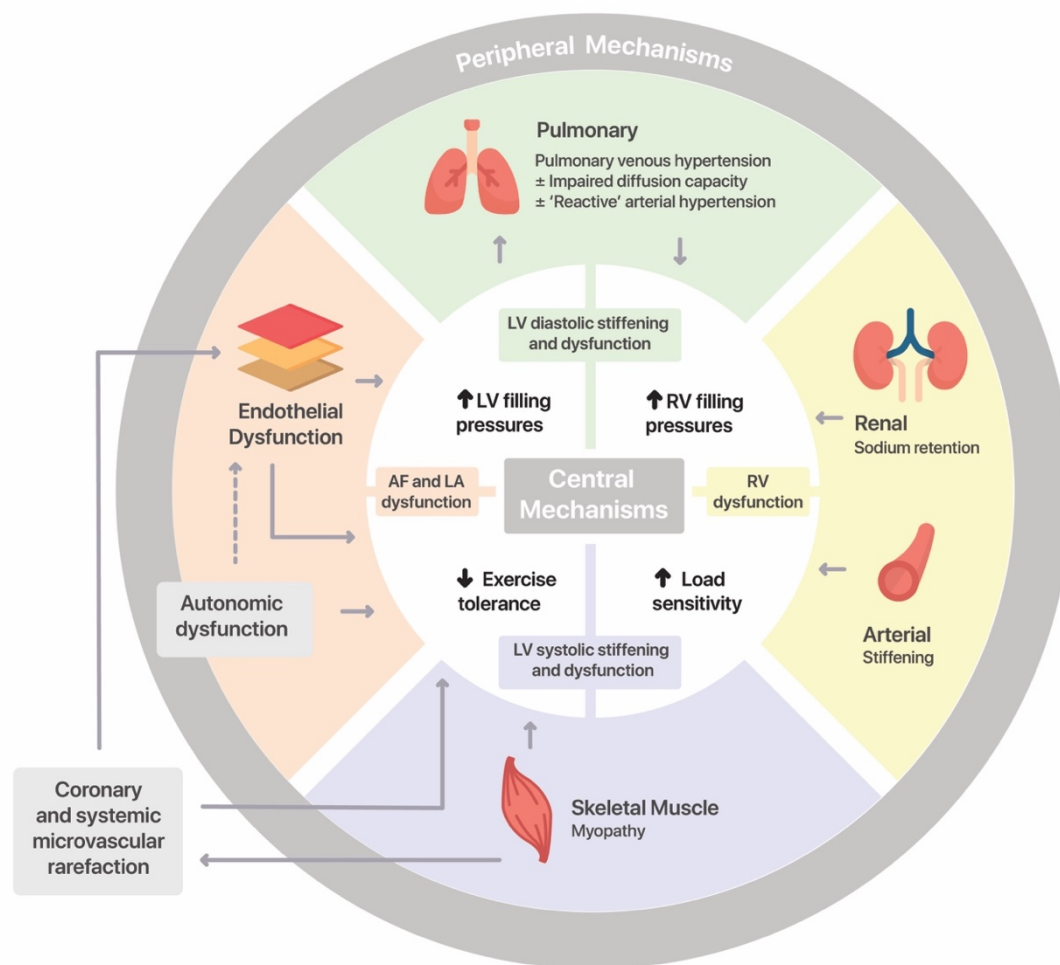


Figure 1: Complex interaction of multiple impairments associated with the heart failure with preserved ejection fraction syndrome. Central mechanisms comprise LV diastolic and systolic stiffening and dysfunction, RV dysfunction, atrial fibrillation and left atrial dysfunction. Peripheral mechanisms comprise impaired pulmonary and renal function, arterial stiffness, abnormalities of skeletal muscle and endothelial dysfunction. AF: atrial fibrillation; LA: left atrial; LV: left ventricular; RV: right ventricular. Adapted from (Zakeri & Cowie, 2018).

The mechanisms underlying HFpEF remain poorly understood. A new paradigm was recently proposed to explain HFpEF (Paulus & Tschope, 2013). According to this hypothesis, low-grade chronic systemic inflammation derived from comorbidities is responsible for coronary microvascular inflammation, which leads to myocardial dysfunction and remodelling (Figure 2). Coronary microvascular endothelial cells produce reactive oxygen species (ROS), which limits nitric oxide (NO) bioavailability for adjacent cardiomyocytes, leading to a downregulation of endothelium-cardiomyocyte signalling [cyclic guanosine

monophosphate content (cGMP) and protein kinase G (PKG) activity] which promotes myocardial hypertrophy and cardiomyocyte stiffness (Franssen et al., 2016). In addition, the increased expression of cell adhesion molecules promotes subendothelial infiltration of circulating monocytes. Monocytes release transforming growth factor β (TGF- β), which promote the differentiation of fibroblasts into myofibroblasts, thus increasing interstitial collagen deposition and interstitial fibrosis. Increased cardiomyocyte stiffness and interstitial fibrosis induce diastolic LV dysfunction (Paulus & Tschope, 2013).

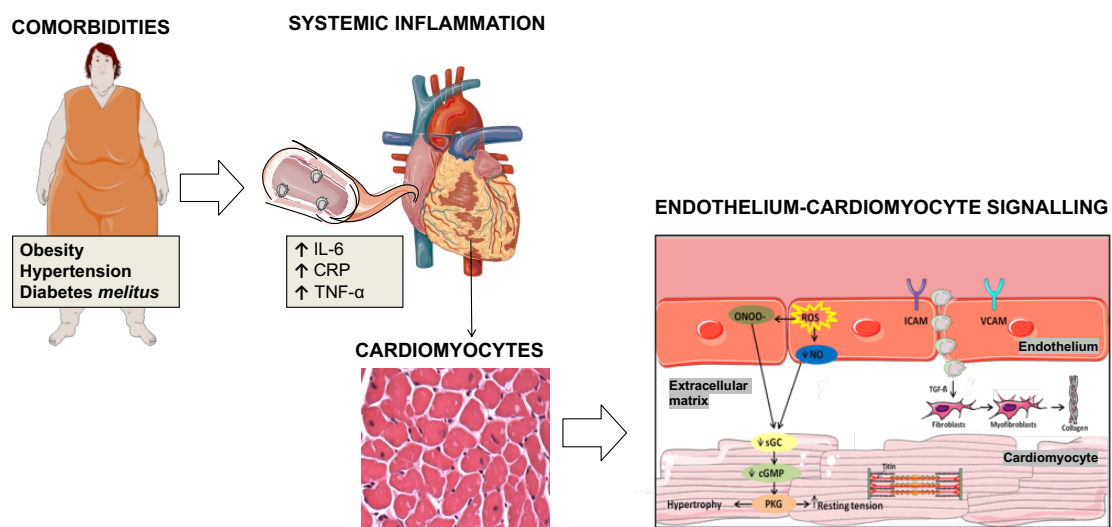


Figure 2: Impact of systemic inflammation on the pathogenesis of HFpEF. Exposure to comorbidities promotes a low-grade systemic pro-inflammatory state that, when sustained, will disturb the coronary microvascular endothelium through the increased production of reactive oxygen species and increased expression of cell adhesion molecules (CAMs). The dysfunctional endothelium affects the signalling from the endothelium to adjacent cardiomyocytes [reduced cyclic guanosine monophosphate content (cGMP) and protein kinase G (PKG) activity] which promotes cardiomyocytes hypertrophy and increase resting tension because of hypophosphorylation of titin. On the other hand, the CAMs promote the infiltration of monocytes, releasing transforming growth factor β (TGF- β), which promote the differentiation of fibroblasts into myofibroblasts, thus increasing interstitial collagen deposition. Overall, increased hypertrophy, cardiomyocyte resting tension and fibrosis will account for cardiac stiffness. IL-6: interleukin-6; CRP: C-reactive protein; TNF- α : tumor necrosis factor alfa; NO: nitric oxide; sGC: soluble guanylate cyclase; ICAM: intercellular adhesion molecules; VCAM: vascular cell adhesion molecules.

2.5. Treatment

According to 2016 ESC guidelines, no therapy has been consistently proven to reduce morbidity or mortality in patients with HFpEF (Ponikowski et al., 2016). However, since these patients are often elders, highly symptomatic, and perceiving a poor quality of life (Fukuta et al., 2016), current treatment ESC guidelines highlight the importance of aiming to alleviate symptoms and improve patients well-being (Ponikowski et al., 2016). Patients should be screened for cardiovascular and non-cardiovascular comorbidities, which if present, should be managed with interventions that have been shown to improve symptoms, well-being or other health-related outcomes, without exacerbating HF (Ponikowski et al., 2016).

Because HFpEF continues to be refractory to available therapies, there is an urgent need for novel approaches not only for the treatment and management of HFpEF but also for its prevention. Indeed, some authors are now arguing that the attention needs to be directed to the early steps of this syndrome, by the screening and management of the potential modifiable contributing factors, ultimately to prevent HFpEF (Bobenko et al., 2018; Kondamudi et al., 2017). In this sense, growing body of evidence is also highlighting the value of physical activity (PA)/exercise training programs in the prevention of HFpEF and in the management of cardinal symptoms such as exercise intolerance and quality of life (Chan et al., 2016). Indeed, the current ESC guidelines highlight the importance of PA/exercise training to HFpEF management (Ponikowski et al., 2016).

3. THE ROLE OF PHYSICAL ACTIVITY IN HFPEF

3.1. Definition of physical activity and sedentary time

Physical activity is usually defined as any body movement produced by skeletal muscles that require energy expenditure (Caspersen et al., 1985). It is commonly categorized according to four different contexts: occupational, household, transport and leisure-time or recreational PA (Physical Activity Guidelines Advisory Committee Scientific Report, 2018). In addition, PA can be

classified by its intensity, such as light [1.5-3 metabolic equivalents (METs)], moderate (3-6 METs), and vigorous or very vigorous intensity (≥ 6 METs) (Riebe et al., 2018). By comparison, exercise training is defined as a sub-component of PA that is characterized to be a deliberated practice, planned, structured, and repetitive, and is designed with the specific purpose of improving or maintaining physical fitness, physical performance, or health (Caspersen et al., 1985). Regarding sedentary time, it is defined as any waking activity characterized by a low level of energy expenditure (≤ 1.5 METs) while sitting, reclining, or lying (Tremblay et al., 2017).

3.2. Measurement of physical activity and sedentary time

As PA is a multidimensional practice, valid measurements in free-living individuals can be a challenging task, regardless the population. There are several methods for measuring PA, and each one has its own particular limitations and strengths. These methods include subjective (e.g. questionnaire) and objective instruments (e.g. accelerometer). Questionnaires are a simple instrument, easily-administered, appropriate to use in large samples for research purposes, and is cost-effective (Besson et al., 2010). In addition, it allows identifying the context in which PA is performed (e.g. occupational and leisure-time or recreational) (Besson et al., 2010). However, the assessment of PA by questionnaires is based on self-reports, and therefore, most often biased due to social desirability, inaccurate memory, and the inability to capture the absolute level of PA intensity (Prince et al., 2008). Accelerometers are motion sensors specially designed to assess PA and related energy expenditure. Measurements with accelerometers provide accurate and reliable information about total PA, as well as about the intensities and time spent at each PA intensity in everyday life activities (Cheung et al., 2011; Gorman et al., 2014). Furthermore, the device size is small and easy to wear. Unfortunately, accelerometers are expensive and challenging because of the large volume of data that generate and/or the required expertise to the data management, which make it difficult to use in large epidemiologic studies (Troiano, 2005).

Sedentary behaviour or sedentary time can also be ascertained from PA questionnaires or accelerometry, respectively. Lower sedentary time is usually related to higher levels of PA in many individuals, however, these variables are not necessarily correlated. In fact, it is somewhat common to find individuals who perform higher levels of PA but, concomitantly, also accumulate higher levels of sedentary time (e.g. sedentary job) (Thorp et al., 2011).

3.3. Physical activity in the prevention of HFpEF

Several studies are supporting the notion that to control the growing burden of HFpEF, we have to change the focus for primary prevention and identify modifiable risk factors that can be targeted. Lifestyle risk factors, such as low PA, sedentary time, and low cardiorespiratory fitness (CRF) are becoming increasingly recognized as major risk factors for most chronic diseases, including HF (Booth et al., 2012; Djousse et al., 2009; Kenchaiah et al., 2009). Indeed, a number of community-based studies have demonstrated an inverse association between PA levels and risk of HF. The Women's Health Initiative, an observational study with a cohort of 84.537 participants, demonstrated that a relatively high levels of PA was associated with decreased HF risk (Agha et al., 2014). Similar results were found in a prospective cohort study using data from 20.900 men, where adherence to healthy lifestyle factors was associated with a lower lifetime risk of HF (Djousse et al., 2009). In addition, physical inactivity (defined as not meeting the recommended amount of health-related PA within a week) was also related to increased risk for HF (He et al., 2001). Young and colleagues examined the correlation between PA and prolonged sedentary time on risk of HF in 82.695 men followed for 10 years (Young et al., 2014). The investigators found that apart from higher PA, lower sedentary time was associated with reduced risk of HF incidence, and these associations had independent contributions (Young et al., 2014).

While it remains to be clarified which HF phenotype is the mostly affected, some reports suggest that PA and sedentary time may be more strongly implicated in the development of HFpEF than in HFrEF. A recent study with a pooled analysis from three large cohorts demonstrated an inverse relationship

between leisure-time PA and HF risk, where lower levels of PA were associated with higher risk of HFpEF but not HFrEF (Pandey et al., 2017). Additionally, in an elderly cohort from the Framingham Heart Study, lower PA levels were more strongly associated with risk of HFpEF (Kraigher-Krainer et al., 2013). Furthermore, corroborating this notion of HF phenotype specificity, it was shown a strong dose-dependent inverse association between lifetime doses of exercise training and LV compliance and distensibility, which are hallmark features of HFpEF (Bhella et al., 2014). Finally, there is evidence that sedentary lifestyle (prolonged bed rest) is also associated with many of the underlying cardiac and skeletal muscle abnormalities often present in HFpEF (Dorfman et al., 2008; Irimia et al., 2017). Thus, it seems that PA and/or sedentary time are important modifiable aspects to be targeted to prevent HFpEF. In addition to that major key point, it needs to be highlighted that the above-mentioned studies support their conclusions on questionnaire-derived information, which often overestimate PA (Lee et al., 2011), and thus do not allow to extract precise data for PA prescription. To overcome this important limitation, objectively measures of PA, such as those derived from accelerometry, need to be explored. Because the use of accelerometers in large cohort studies is not feasible, an alternative approach may be the evaluation of physical fitness since it can be relatively easily assessed and is strongly related to PA. Recent work from the Cooper Centre Longitudinal Study showed that in midlife, lower CRF, which is one component of physical fitness, is more powerfully associated with a marked increase in the risk for HF than acute myocardial infarction in older age (Berry et al., 2013). In addition, lower CRF in midlife was related to a larger degree of diastolic dysfunction and LV remodelling, important hallmarks of HFpEF (Brinker et al., 2014). Similar risk patterns were recently observed by Pandey and co-workers, where lower CRF in young adulthood was strongly associated with subclinical diastolic filling impairment 20 years later (Pandey et al., 2017a). Finally, CRF was inversely associated with relative wall thickness and LVMI in adults, both hallmarks of HFpEF (Lam et al., 2010). Collectively, these studies suggest that higher levels of PA, less time spent in sedentary behaviours and a greater CRF are strongly associated with a reduced risk of HFpEF.

3.4. Physical activity in the management of HFpEF

The role of PA/exercise training programs on the management of HFpEF is gaining special attention, with published data suggesting their benefits on several clinical outcomes with important prognostic implication. In the next section, we will review the main findings regarding outcomes as hospitalization and mortality, exercise capacity, quality of life, cardiac function and remodelling, and biochemical markers of biological processes include in HFpEF.

3.4.1. Hospitalization and mortality

A dose-response relationship between PA levels and risk of adverse outcomes, such as HF hospitalization, have been well described in patients with HFrEF (Sagar et al., 2015). However, the impact of increasing PA levels in those with HFpEF is less clear. Recent evidence from the TOPCAT study, with a cohort of 1.751 individuals, showed that higher levels of PA among stable patients with HFpEF were associated with a reduced risk of adverse outcomes, including hospitalization and cardiovascular mortality (Hegde et al., 2017). The reduced risk of adverse outcomes may be related to better indices of diastolic function, as previously noted in other cohort studies with 2.925 individuals (Brinker et al., 2014). It is important to note that in the TOPCAT study, the authors observed a dose-response relationship between PA levels and risk of adverse clinical outcomes, such that only PA levels at or above current recommended guidelines (150 min/week of moderate activity, or at least 75 min/week of vigorous activity, or 150 min/week of a combination of both) were associated with a lower risk of hospitalization or mortality (Hegde et al., 2017). These data suggest that a higher dose of PA may be required to achieve benefits regarding hospitalization and mortality. However, since the TOPCAT study relied on PA measurements by questionnaire, it should be viewed with wariness. For a better understanding of the impact of PA in HF hospitalization and mortality, a more objective method for PA quantification, such as PA actigraphy using accelerometers, should be used. Regarding structured exercise training, while RCTs support its safety (Chan et

al., 2016), the effect on mortality or hospitalization is largely unknown and future studies need to specifically address these endpoints.

3.4.2. Exercise capacity

Several studies demonstrated significant improvements in exercise capacity with PA and exercise training interventions in patients with HFpEF. A multicentre study from the NEAT-HFpEF trial evaluated daily PA levels using accelerometers in 110 patients with HFpEF (Snipelisky et al., 2017). The authors reported a significant association between PA levels and exercise capacity assessed through the 6-minute walk test (6MWT), where patients with lower levels of daily PA had lower exercise tolerance. This was corroborated in the multicentre ALDO-DHF trial with 442 ambulatory HFpEF patients, where the total amount of PA (METs/week) was positively correlated with submaximal exercise capacity (Bobenko et al., 2018). Indeed, HFpEF patients have a significant reduction in exercise capacity (Dhakal et al., 2015), which can lead to early fatigue and reduction in the overall volume of daily activity. In comparison with healthy individuals at risk of HF, and HFrEF patients, it seems that patients with HFpEF have the lower volume of objectively measured daily PA (Yavari et al., 2017).

Moreover, a positive effect of exercise training on exercise capacity was also shown by a meta-analysis addressing large HFpEF trials (Chan et al., 2016). From the 8 studies included in the final analysis, 5 showed a significant improvement in peak oxygen consumption (VO_{2peak}) ($+2.08 \text{ ml.kg}^{-1}.\text{min}^{-1}$; 15% increase), which is above the clinically meaningful change ($1 \text{ ml.kg}^{-1}.\text{min}^{-1}$ or 10%) in VO_{2peak} for patients with HF (Kitzman, 2011). In addition, 4 studies reported a significant increase in walking distance on the 6MWT ($+32.1$ meters). Of note, these improvements were obtained with different types of exercise. Just to mention some, Haykowsky and colleagues performed 4 months of endurance exercise training in HFpEF patients and found an improvement of $2.3 \text{ ml.kg}^{-1}.\text{min}^{-1}$ on VO_{2peak} (Haykowsky et al., 2012). In addition, a multicentre RCT compared 3 months of combined endurance and strength training with usual care alone in 64 clinically stable patients with HFPEF. Peak VO_2 increased with exercise training (from $16.1 \pm 4.9 \text{ ml.kg}^{-1}.\text{min}^{-1}$ to $18.7 \pm 5.4 \text{ ml.kg}^{-1}.\text{min}^{-1}$; $p < 0.001$), while it

remained unchanged with usual care alone (Edelmann et al., 2011). Finally, aerobic interval training was also effective to significantly improve VO_2 peak in HFpEF patients (Fu et al., 2016) as well as to improve exercise tolerance above the threshold considered clinically relevant (Alves et al., 2012). Interestingly, a recent meta-analysis showed that the single use of standard cardiovascular medications failed to improve exercise capacity, while its combination with exercise training was effective to increase VO_2 peak and the performance in 6MWT (Fukuta et al., 2016).

The physiological mechanisms underlying these improvements in exercise capacity in HFpEF patients are poorly comprehended. Poor exercise response was shown to be due to a reduction in both cardiac output and arteriovenous oxygen content difference (Houstis et al., 2018). Therefore, by knowing the effects of exercise training in other clinical settings, it seems logical to hypothesize that training would improve exercise capacity by influencing both factors. However, current data supports that the greater VO_2 peak found in HFpEF patients after training is paralleled by an increase in oxygen extraction but not by cardiac output (Fu et al., 2016; Haykowsky et al., 2012). Nevertheless, it needs to be highlighted that the number of studies assessing cardiac hemodynamic after training is very low and the number of recruited patients is reduced (Fu et al., 2016; Haykowsky et al., 2012). Moreover, these studies were not designed to test the most effective dose to potentially improve hemodynamic factors, and it may be the case that cardiac responsiveness to training is reduced and requires prolonged (several months to years) and/or more intensity of training programs as recently suggested for healthy seniors (Bhella et al., 2014). Indeed, it was shown that competitive master athletes demonstrated improved ventricular compliance (lower LV chamber stiffness constants) and distensibility (greater LVEDVi) compared to that in casual exercisers and sedentary subjects (Bhella et al., 2014). Finally, because HFpEF patients are so heterogeneous, it may be the case that the magnitude of responsiveness of each factor to training is dependent on the patient's personal profile of defects (Houstis et al., 2018). Figure 3 summarizes how these factors could cooperate to improve exercise capacity in HFpEF.

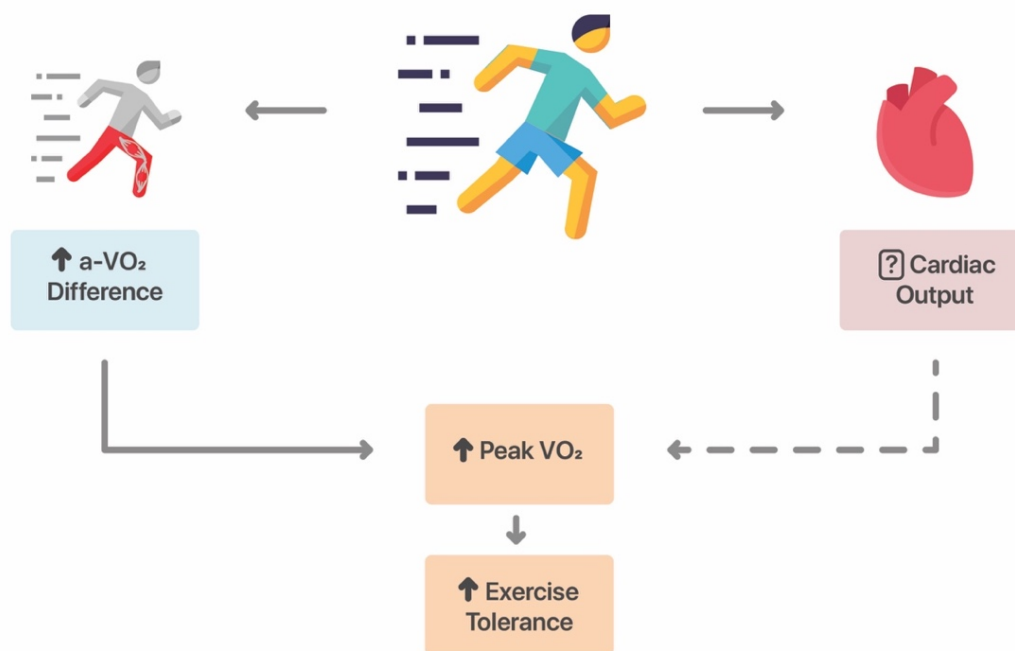


Figure 3: Potential mechanisms by which exercise training might improve VO₂peak in HFpEF. Current evidences suggest that an increase in oxygen extraction but not in cardiac output is the main responsible for improved VO₂ peak. Dotted grey line means that there is no evidence of improved cardiac output in HFpEF patients. However, only 2 studies with a small number of patients assessed cardiac hemodynamic. More evidences are needing to better understand the contribute of both factors.

3.4.3. Quality of life

Health-related quality of life has been considered another very important outcome for HFpEF patients. Higher levels of PA were related with better quality of life measured by the Kansas City Cardiomyopathy Questionnaire (Hegde et al., 2017; Snipelisky et al., 2017). Regarding to exercise training, most of the studies assessed quality of life by the Minnesota Living with Heart Failure Questionnaire (MLHFQ) (Rector & Cohn, 1992). This questionnaire was developed to assess the patient's perception of the impact of the disease and its treatment on their life, and it reflects the general, physical and the emotional dimensions of quality of life. In a recent meta-analysis, Chan and colleagues evaluated the effects of exercise training on quality of life in 317 participants with HFpEF (174 exercising and 143 control) (Chan et al., 2016). The authors reported that 7 out of 8 studies showed significant improvements in general MLHFQ score (mean difference of -6.77 points). Nevertheless, the authors did not perform any analysis by each dimension (emotional and/or physical). However, it seems that

the large majority of the studies using the MLHFQ (and reporting the dimensions) showed an improvement only in the physical dimension of quality of life (Edelmann et al., 2011; Kitzman et al., 2010). This may be explained by the attenuating effect of exercise training over exercise intolerance and fatigue, increasing patients' ability to cope with the demands of daily tasks, and consequently improving the perceived quality of life in the physical dimension (Fu et al., 2016). Finally, Smart et al. (2007) showed that 16 weeks of exercise training only significantly improved the emotional dimension in HFpEF patients (Smart et al., 2007). Taken together, these evidences suggest that exercise training programs are effective strategies to induce positive effects on general quality of life.

3.4.4. Cardiac function and remodelling

Despite the overall beneficial effects of increasing levels of PA in HFpEF patients, it remains poorly understood if it can also modulate cardiac function and remodeling. Some preliminary clues to this aspect are provided by a recent study showing that HFpEF patients engaged in lower levels of daily PA (objectively measured by accelerometry) presented higher NT-proBNP levels, more concentric left ventricular remodeling (higher relative wall thickness), larger LAVi, and a tendency to have higher E/e' (Snipelisky et al., 2017). Contrasting findings were shown by Bobenko and colleagues, who did not find association between E/e' or LAVi and PA levels in HFpEF patients. However, since in the last study PA levels were based on self-report, the results should be viewed with wariness (Bobenko et al., 2018).

Regarding to the impact of exercise training on diastolic function in HFpEF, early meta-analysis suggested that exercise training was not associated with significant changes in diastolic function (Pandey et al., 2015). This lack of evidence could be due to the different standard measures of diastolic function (E/A ratio and deceleration time) between studies included in the meta-analysis. Moreover, the E/e' is considered a more specific non-invasive indicator of diastolic function, once that it is a less load dependent index of LV relaxation and less influenced by heart rate or age (Pearson et al., 2017). However, studies

using this parameter were scarce at that time (n=1) and thus were excluded from meta-analysis. In this sense, a more recent meta-analysis of 8 studies, with 317 participants, showed that exercise training programs significantly improve diastolic function (changes on E/e', E/A and/or deceleration time) (Chan et al., 2016). These results differ from the meta-analysis aforementioned because it included more studies that evaluated E/e'. Future studies using invasive assessment (catheterization) of cardiovascular properties at rest and during exercise may provide a more sensitive analysis of alterations induced by exercise training (Borlaug & Kass, 2009). In the meantime, improvement of diastolic function with exercise training was also demonstrated in pre-clinical studies (Hidalgo et al., 2014; Slater et al., 2017).

Regarding the underlying mechanisms, so far, the literature does not elucidate how exercise training could improve diastolic function in HFpEF patients. Edelmann and colleagues demonstrated that exercise training improves LV diastolic function and atrial reverse remodelling without change in LV mass, suggesting a switch from pathologic into a more physiologic hypertrophy (Edelmann et al., 2011). Indeed, exercise training has the ability to counterbalance the structural and functional cardiac changes induced by cardiovascular diseases, contributing to phenotypical changes of pathological cardiac hypertrophy into physiological cardiac hypertrophy (Fernandes et al., 2015). In addition, exercise training was associated with a significant reduction in procollagen type I plasma levels, suggesting that improvement in diastolic function may be associated with reduced collagen turnover (Edelmann et al., 2011). NT-proBNP is the gold standard marker for myocyte stress and the decreases in NT-proBNP levels were associated with reduced mortality and morbidity rates in HFpEF patients (Anand et al., 2011). While exercise training was capable to significantly reduce the circulating concentrations of NT-proBNP (Conraads et al., 2004) or BNP (Nakanishi et al., 2017) in HFrEF patients, no significant changes have been noted in patients with HFpEF (Edelmann et al., 2011; Kitzman et al., 2010). However, it is important to highlight that levels of NTproBNP (Edelmann et al., 2011) or BPN (Kitzman et al., 2010) in these studies were below the levels that were reported in acutely decompensated HFpEF.

Finally, whether exercise training can modulate LV compliance and distensibility in HFpEF remains to be explored, but these diastolic variables were recently shown to be modulated by exercise training in healthy middle age individuals (Howden et al., 2018) and in healthy seniors (Bhella et al., 2014).

Further insights of the molecular mechanisms underlying exercise-induced benefits in HFpEF are provided by animal models. Pre-clinical studies of HFpEF suggest that exercise-induced improvement on diastolic function are linked to decreased myocardial stiffness (Figure 4). A recent work evaluated the effect of free-wheel running exercise on cardiac stiffness (extracellular matrix and titin-based stiffness) in a genetic mouse model ($Ttn\Delta I\alpha_{jxn}$), where the I-A junction of titin is removed resulting in increased diastolic stiffness and reduced exercise tolerance (Slater et al., 2017). The data revealed that intrinsic myocardial stiffness was reduced in the running group, which was associated with reduced titin-based passive stiffness in a phosphorylation-dependent manner, as no effect was detected on extracellular matrix (Slater et al., 2017). The authors observed that the reduction in passive stiffness was mainly related to both decreased PEVK phosphorylation and increased N2B phosphorylation (Slater et al., 2017). The phosphorylation of PEVK element by protein kinase C α (PKC α) increases titin stiffness, while phosphorylation of N2B element by protein kinase A (PKA) or protein kinase G (PKG), decreases titin stiffness (Kotter et al., 2013). Voluntary exercise in genetically engineered mice with HFpEF symptoms (IG KO mice), was shown to decrease PKC α expression level in the LV, lowering titin-based stiffness and improving diastolic filling (Hidalgo et al., 2014). In addition, Slater and colleagues reported that voluntary exercise in $Ttn\Delta I\alpha_{jxn}$ mice increases titin phosphorylation by PKA, leading to diminished titin stiffness and improved diastolic function (Slater et al., 2017). These studies suggest that reduced passive tension induced by exercise appears to be explained mainly by modification of cardiac titin phosphorylation status, rather than by titin isoform expression. Indeed, these experimental studies did not found changes in the ratio of the N2B or N2BA titin isoforms (Hidalgo, Saripalli, & Granzier, 2014; Slater et al., 2017).

At the level of extracellular matrix, treadmill exercise training was shown to attenuate diastolic impairment by reducing fibrosis in the Yucatan miniature swine model of HFpEF (aortic-banded to produce concentric LV hypertrophy) (Marshall et al., 2013). The reduction of fibrosis was associated with normalization in the relative mRNA levels of matrix metalloproteinases (MMPs) (MMP-2 and MMP-9), and their tissue inhibitors (TIMPs) (TIMP-1 and TIMP-4). Modification on the collagen degradation system was shown to be an important contributor to alterations on extracellular matrix, which can lead to diastolic dysfunction (Westermann et al., 2011).

While the upstream modulators of this beneficial effects are also far from being comprehended, it can be hypothesized that the systemic effects promoted by exercise training may interfere with the cascade of events leading to cardiac stiffness and HF development. Indeed, previous data from different clinical conditions provides evidence that exercise training has anti-inflammatory (Smart & Steele, 2011) and anti-oxidative proprieties (Sties et al., 2018) and improves endothelial function (Pearson & Smart, 2017), all of which are thought to be implicated in the pathophysiology of HFpEF (Paulus & Tschope, 2013).

Despite these molecular insights provided by pre-clinical studies, it is important to highlight that none of these animal models fully mimic the spectrum of changes found in humans with HFpEF. In addition, in humans, HFpEF is a condition typically associated to underlying comorbidities and exercise intolerance, while development of these conditions is rather rare in most animal models (Lourenco et al., 2018). In an attempt to surpass these limitations, a new HFpEF model using the obese ZSF1 rat has been proposed (Hamdani et al., 2013). It is considered a robust model as these animals display hypertension, obesity, type 2 diabetes, insulin resistance, hyperinsulinemia, hypertriglyceridemia and hypercholesterolaemia (Hamdani et al., 2013). Over time, this cardiometabolic risk model develops the main features found in humans diagnosed with HFpEF: i) reduced exercise tolerance and VO_{2peak} , ii) preserved systolic function (LVEF, LV maximum rate of pressure rise, and the slope of linear end-systolic pressure-volume relationship for indexed volumes) and iii) diastolic dysfunction (higher E/e' , increased left atrial area, prolonged tau, elevated left

ventricular end-diastolic pressure, an upwards shift of LV diastolic pressure-volume relationship, and higher LV diastolic chamber stiffness constant) (Hamdani et al., 2013; Leite et al., 2015).

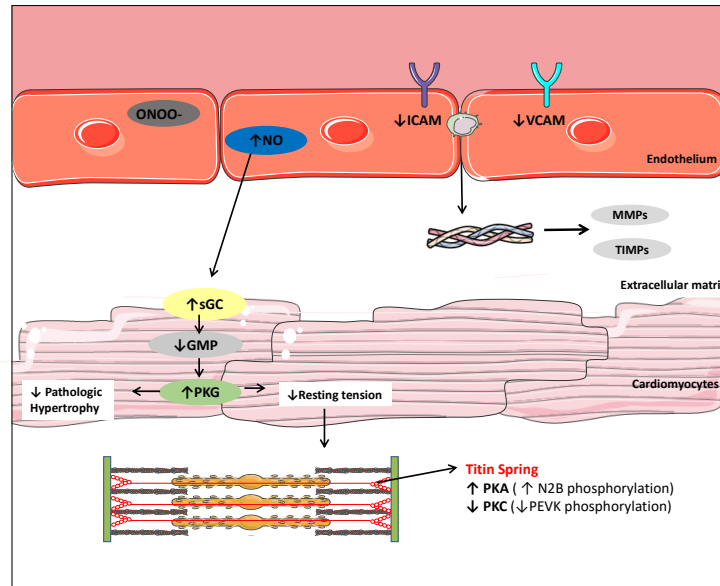


Figure 4: Effects of exercise training on myocardial stiffness. Exercise training can reduce titin-based passive stiffness and LV fibrosis. It is related with decrease protein kinase (PK) C α (PKC α) expression and increases PKA, which leads to both decreased PEVK phosphorylation and increased N2B phosphorylation, respectively. Reduced fibrosis may be mediated by normalization in the relative mRNA levels of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs), reducing the producing of collagen. ONOO: peroxynitrite, NO: nitric oxide, sGC: soluble guanylate cyclase; GMP: guanosine monophosphate content; ICAM: intercellular adhesion molecules; VCAM: vascular cell adhesion molecules.

4. PHYSICAL ACTIVITY/EXERCISE PRESCRIPTION FOR MANAGEMENT OF HFPEF

The optimal dose of PA that is beneficial to improve adverse outcomes in HFpEF patients is unknown. The current American Collage of Sport and Medicine (ACSM) PA guidelines recommend for older adults a minimum of 150 min/week of moderate activity, or at least 75 min/week of vigorous activity, or 150 min/week of moderate plus vigorous activity, to promote health and reduce the risk of chronic disease (Physical Activity Guidelines Advisory Committee Scientific Report, 2018). However, the current guidelines were not specifically tailored to patients with established HFpEF. It is recognized that there is an inverse dose-response relationship between PA and health-related benefits (Hegde et al.,

2017). Indeed, recent findings shown that only the highest PA intensities measured by accelerometers were associated with maximal exercise capacity (Bobenko et al., 2018). However, as Yavari and colleagues observed, for the majority of patients with HFpEF it is difficult to achieve the minimum recommended amount of moderate or vigorous PA per week (Yavari et al., 2017). In this sense, it is important to highlight that the ACSM PA guidelines make clear that any increase in the amount of PA, regardless the intensity, translates into health benefits (Physical Activity Guidelines Advisory Committee Scientific Report, 2018). In the clinical context, patients should be encouraged to increase the daily total time spent at moderate or vigorous PA and progressively increase the frequency and duration at this intensity.

Regarding exercise training, although ESC guidelines recommend that HFpEF patients should perform properly designed exercise training (Ponikowski et al., 2016), there is no specific recommendations regarding the type, doses or intensity of exercise that can be followed to optimize the management these patients. While this work was not designed to systematically review the literature in order to be able to suggest which is the most effective exercise training program, it seems that the type of exercise is an important “ingredient” to positively modulate the main clinical outcomes in HFpEF. In fact, combined endurance and resistance training for 3 months (2-3 days/week) (Edelmann et al., 2011), as well 6 months (2–3/week) (Nolte et al., 2014) were shown to improved functional capacity, quality of life and diastolic function in HFpEF patients. In addition, aerobic interval training performed 3 times per week for 12 weeks (Fu et al., 2016) or 6 months (Alves et al., 2012) was also able to improve functional capacity and diastolic function. Quality of life was also improved by the former cited study (Fu et al., 2016). Conversely, moderate aerobic exercise training, performed 3 times per week for 16 weeks (Haykowsky et al., 2012; Kitzman et al., 2010; Smart et al., 2012) or 1 year (Fujimoto et al., 2012) was not effective to improve diastolic function in HFpEF patients. In addition, inspiratory muscle training for 12 weeks was able to improve exercise capacity and quality of life, but no changes on diastolic function was observed (Palau et al., 2014). In summary, combined exercise training or aerobic interval training seems to be the

better type of exercise to improve exercise capacity, quality of life and diastolic function in HFpEF. These types of exercise training may be particularly important for older age or frail patients who may not be able to tolerate continuous aerobic exercise. Three times per week, for 12 weeks was shown to be sufficient to induce these benefits. However, the necessary amount of exercise training to maintain these benefits is unknown.

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CHAPTER II

GAPS AND AIMS

From the above brief review, some gaps in the literature were identified, which leverage the original studies encompassing this thesis. First, previous evidence shows that lower levels of physical fitness are associated with poor quality of life in patients with HFpEF (Kitzman et al., 2010; Nolte et al., 2015), but, up to date, only CRF was associated with a better quality of life, specifically with the physical dimension (Edelmann et al., 2011; Kitzman et al., 2010). However, physical fitness is multicomponent construct (e.g. dynamic balance and mobility, muscular fitness, CRF and body composition) (Rikli & Jones, 2013) and quality of life is multidimensional (e.g. general, emotional and physical) (The World Health Organization Quality of Life Assessment Group, 1998). Therefore, it remains unknown whether other physical fitness components impact on dimensions of quality of life, in HFpEF patients. Better clarification of this issue might have important clinical implications in the design of specific interventional programs for HFpEF patients by targeting the physical fitness component that mostly impacts quality of life. Therefore, the first aim of the thesis was:

STUDY 1:

- a) to examine the association between different components of physical fitness (CRF, upper body strength, dynamic balance and mobility, and body composition) and the dimensions of quality of life (total, physical and emotional) in HFpEF patients;
- b) to examine which of the physical fitness components are independently related to different dimensions of quality of life in this specific population.

Second, in HFpEF patients there is a lack of studies describing and comparing daily PA levels assessed by questionnaire and accelerometers. There is a growing recognition of the important role that PA can have on the management of HFpEF (Hegde et al., 2017). From a clinical point of view accurate and reliable methods to assess PA are needed to provide tailored counselling and prescription. Therefore, the second aim of the thesis was:

STUDY 2:

- a) to determine the validity of the International Physical Activity Questionnaire (IPAQ) against objective measures from triaxial accelerometry in HFpEF patients;
- b) to describe patterns of daily PA and sedentary time measured by accelerometry;
- c) to study the association of objective PA measures with prognostic indicators.

Third, although there is some evidence that exercise training can improve diastolic function in HFpEF patients, the mechanisms underlying this improvement are still unknown. In the impossibility to obtain human cardiac biopsies, pre-clinical models are the preferred choice to address this issue. However, given the complex pathophysiology underlying HFpEF, none of the current models fully mimic the human phenotype. The ZSF1 obese animal model was shown to present several features of HFpEF (Hamdani et al., 2013; Leite et al., 2015), placing this model one step ahead towards clinical translation (Lourenco et al., 2018). However, to our best knowledge, there are no studies evaluating the impact of exercise training in HFpEF using this model. Understanding the exercise-induced adaptations may hold promise as potential targets and open the door to novel therapeutic approaches aimed at restoring diastolic function. Therefore, the third aim of the thesis was:

STUDY 3:

- a) to examine the effects of exercise training on diastolic function in the ZSF1 obese animal model of HFpEF;
- b) to analyse structural and molecular changes induced by exercise training.

To accomplish the first and second aims, a cross-sectional study with HFpEF patients was conducted in a Portuguese public hospital (*Centro Hospitalar do Porto - Hospital de Santo Antonio, Porto*). Patients were recruited

from November 2016 to September 2017. All data from each patient was collated in the same day. The obtained data are presented in **Study I** and **Study II** in the next section. To accomplish the third aim, a low-intensity exercise training protocol using the ZSF1 obese animal model was conducted, which gave origin to our **Study III**.

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CHAPTER III

ORIGINAL STUDIES

STUDY I

DYNAMIC BALANCE AND MOBILITY EXPLAINS QUALITY OF LIFE IN HEART FAILURE WITH PRESERVED EJECTION FRACTION, OUTPERFORMING ALL THE OTHER COMPONENTS OF PHYSICAL FITNESS

ABSTRACT

Rational: Physical fitness is an important determinant of quality of life (QoL) in heart failure with preserved ejection fraction (HFpEF) patients. However, physical fitness and QoL are multicomponent and multidimensional, respectively, remaining unknown how the different components of physical fitness relate with the specific dimensions of QoL in HFpEF patients.

Aim: To evaluate the association between different components of physical fitness and dimensions of QoL in HFpEF patients, and, to examine which physical fitness components are independently related to different dimensions of QoL.

Methods: Patients with HFpEF (n=24; stable and well-medicated) were recruited and assessed for physical fitness [dynamic balance and mobility (8-feet-up-and-go test), upper body strength (handgrip strength), cardiorespiratory fitness (CRF) (6-minute-walking test) and body composition (body mass index)] and for QoL (Minnesota Living With Heart Failure Questionnaire). The dimensions of QoL were total score, emotional and physical. Partial correlation was used to verify the association between physical fitness components and dimensions of QoL. The determination of independent predictors dimensions of QoL was computed through stepwise multivariate linear regression analysis.

Results: Both CRF and dynamic balance and mobility are significantly associated with total score and physical dimensions of QoL ($p < 0.05$), but only dynamic balance and mobility was concomitantly associated with the emotional dimension ($r = 0.597$; $p = 0.004$). Dynamic balance and mobility was independently associated with total score ($\beta = 0.651$; $r^2 = 0.424$; $p = 0.001$), physical ($\beta = 0.570$; $r^2 = 0.324$; $p = 0.04$) and emotional ($\beta = 0.611$; $r^2 = 0.373$ $p = 0.002$) dimensions of QoL. Upper body strength, CRF, body composition were not independent predictors of any dimensions of QoL ($p > 0.05$).

Conclusion: Our data suggests that dynamic balance and mobility better capture QoL than the commonly measured in clinical practice CRF. Whether interventions specifically targeting dynamic balance and mobility differently impacts on QoL is still unknown.

Keywords: physical fitness, quality of life, dynamic balance and mobility, HFpEF.

INTRODUCTION

Heart failure with preserved ejection fraction (HFpEF) accounts for half of all HF population in the developed world (Dunlay et al., 2017). The most common manifestation of the disease is exercise intolerance, which impacts on patients' ability to cope with activities of daily life and reduces their quality of life (QoL) (Edelmann et al., 2011b). Furthermore, QoL is related with poor outcomes such as higher frequency of hospital readmission and higher mortality rates (Rodriguez-Artalejo et al., 2005). Despite its high prevalence and poor prognosis, HFpEF remains a disease with no approved therapy that improves survival (Holland et al., 2011). Therefore, current recommendations for the treatment of these patients highlight the importance to focus on effective therapies capable to alleviate symptoms and meaningfully improve QoL (Ponikowski et al., 2016).

Reduced levels of physical fitness are associated with poor QoL in patients with HFpEF (Kitzman et al., 2010; Nolte et al., 2015). Importantly, exercise training was shown to improve physical fitness, together with an amelioration in symptoms and QoL (Kitzman et al., 2010; Nolte et al., 2015). Because physical fitness and QoL are mutually related, targeting physical fitness with exercise training programs may be an effective strategy to accomplish the management recommendations of patients with HFpEF (Ponikowski et al., 2016). However, physical fitness is multicomponent construct (e.g. dynamic balance and mobility, muscular fitness, cardiorespiratory fitness (CRF) and body composition) (Rikli & Jones, 2013) and, in parallel, QoL is multidimensional (e.g. general, emotional and physical) (The World Health Organization Quality of Life Assessment Group, 1998). Until now, it remains unknown how the different components of physical fitness relate with the specific dimensions of QoL in HFpEF patients. To date, it was only demonstrated that higher CRF is associated with a better QoL, mainly the physical dimension (Edelmann et al., 2011a; Kitman et al., 2010). However, the influence of other physical fitness components on QoL dimensions is relatively unknown. Therefore, the clarification of this issue might have important clinical implications in the design of specific interventional programs for HFpEF patients targeting the physical fitness component that most impacts QoL in general or in one of its depressed dimensions.

Therefore, the aims of the present study are twofold: i) to evaluate the association between different components of physical fitness (CRP, upper body strength, dynamic balance and mobility, and body composition) and the dimensions of QoL (total, physical and emotional) in HFpEF patients, and ii) to examine which of the physical fitness components are independently related to different dimensions of QoL in this specific population.

MATERIAL AND METHODS

Study design, recruitment and participants

This is a cross-sectional study conducted in a Portuguese public hospital (*Centro Hospitalar do Porto - Hospital de Santo Antonio*, Porto). Inclusion criteria was diagnosis of HFpEF according to the European Society of Cardiology guidelines (McMurray et al., 2012). Patients were excluded if they presented with unstable angina, acute coronary syndrome as primary diagnosis, symptomatic severe aortic stenosis, acute pulmonary embolus, acute myocarditis, uncompensated heart failure, uncontrolled hypertension, complex ventricular arrhythmias, severe renal dysfunction, severe chronic obstructive pulmonary disease, medical or orthopaedic conditions that precluded independent ambulation and exercise testing.

Patients who potentially were eligible to participate in the study were identified from the clinical files of the hospital's cardiology department. A total of 30 patients were invited through phone calls by a cardiologist. From those, 24 patients (17 women and 7 men) accepted to take part in the study. The study was approved by the Ethics Committee of *Centro Hospitalar do Porto - Hospital de Santo Antonio* (N/S: 2015.125). All procedures were conducted according to the declaration of Helsinki, and participants signed informed consent to participate

Data were collected from November 2016 to September 2017 in a single day in the hospital.

Data collection

Blood pressure

A trained researcher performed blood pressure measurements after 10 minutes resting in seated position. Blood pressure was assessed (Colin, BP 8 800; Critikron, Inc., USA) in the left arm and systolic blood pressure and diastolic blood pressure were computed as the average of 3 readings. Additional readings were performed when differences between readings exceeded 5 mmHg (Mancia et al., 2014).

Blood collection and biochemical determinations

Peripheral venous blood (15 mL) was collected to EDTA tube. The EDTA tubes were immediately placed on ice and allowed to clot for 30 minutes before centrifugation for 15 minutes at 1000xg. The plasma was aliquoted and stored at -80°C for biochemical analysis. Brain Natriuretic Peptide (BNP) was quantified in a certificated laboratory by chemiluminescent microparticle immunoassay (ARCHITECT BNP).

Anthropometric and body composition measures

Body height (cm) was measured standing upright against a stadiometer (Holtain Ltd., Crymmych, UK) (Lohman, 1988). Patients were bare or stocking feet. Weight (kg), body mass index (BMI; kg/m²), fat mass (%) and free fat mass (kg) were measured with patients lightly dressed, using an electronic segmental body composition analyser (Tanita, BC-418, Tokyo, Japan). Waist circumference (cm) was measured at the midpoint between the lowest rib and the iliac crest at the end of normal expiration (Riley et al., 2016). Obesity was determined as BMI equal or higher than 30 kg/m² (WHO, 2000).

Functional classification

Patients were classified by the physician into subgroups based on their symptoms using the New York Heart Association (NYHA) functional class. Patients symptoms is based on how much they are limited during physical activity (class I to IV) (Dolgin, 1994).

Echocardiography Evaluation

Supine transthoracic echocardiography was performed using a cardiovascular ultrasound Vivid E95 ® (GE Healthcare). All quantitative echocardiographic measurements were performed by a single reader blinded to the results of the other evaluations, using a computerized off-line analysis station. Peak early diastolic tissue velocity was measured at the septal and lateral mitral annulus. Mitral inflow velocity was assessed by pulsed wave Doppler from the apical 4-chamber view, positioning the sample volume at the tip of the mitral

leaflets. E/e' ratio was calculated as E wave divided by e' velocities. LV mass was estimated from LV linear dimensions and indexed to body surface area as recommended by ESC guidelines (Lang et al., 2015). LV hypertrophy was defined as LV mass indexed to body surface area (LVMI) >115 g/m² in men or >95 g/m² in women. LV volumes were estimated by the modified Simpson method using the apical 4- and 2-chamber views, and LVEF was derived from volumes in the standard manner. LA volume was estimated by the method of disks using apical 4- and 2-chamber views at an end-systolic frame preceding mitral valve opening and was indexed to body surface area to derive LA volume index.

Physical Fitness

Dynamic balance and mobility

Dynamic balance and mobility was assessed with the 8-foot up and go (8FUG) test (Jones, 2002). Patient starts the evaluation in the seated position. After a signal, patient must stand up, walk 8 feet (2,44m), turned around a cone, and return back to the initial position as fast as possible (Jones, 2002). Patients had two attempts. Time (in seconds) to complete each trial was measured with a stopwatch and the result was the shorter trial (Jones, 2002).

Upper body strength

Grip strength (kg) was isometrically measured using a Lafayette Instrument Hand dynamometer (*Model 78010, 78011, Indiana, USA*). Both arms were measured 3 times while patients were seated, with shoulder adducted and neutrally rotated, the elbow flexed at 90°, and the forearm and wrist in neutral position. The average between attempts was used as final score for each arm (MacDermid et al., 2015).

Cardiorespiratory fitness with pulmonary gas exchange assessment

Cardiorespiratory fitness was assessed by the 6-minute walk test (6MWT) in a 25-m-long unobstructed corridor. Participants were instructed to walk the maximal distance in 6 minutes time. Resting stops were allowed when patients

feel to be necessary. The 6MWT was performed wearing a portable gas analyser (K4b2, Cosmed, Rome, Italy) and a heart rate monitor (Polar Electro Oy, Kempele, Finland). Oxygen uptake (VO_2 ; $\text{mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$) and heart rate (HR; bpm) were measured directly and continuously. In addition, minute ventilation (VE ; $\text{L}\cdot\text{min}^{-1}$), absolute and relative VO_2 , carbon dioxide output (VCO_2 ; $\text{mL}\cdot\text{min}^{-1}$), ventilatory equivalents for oxygen (VE/VO_2), ventilatory equivalents for carbon dioxide output (VE/VCO_2) and respiratory gas exchange ratio (RER) were collected. Respiratory and HR samplings were collected in a breath-by-breath and beat-to-beat basis, respectively, and then, data were averaged over 5-s intervals. Data was calculated as the averaged of measures taken in test total duration (6 minutes).

Health-related quality of life

Health-related QoL was performed by interview through the Minnesota Living With Heart Failure Questionnaire (MLWHFQ). The MLHFQ encompasses 21 questions, whose purpose is to determine how disease affects the physical, psychological and socioeconomic conditions of the patients during the previous month (Rector & Cohn, 1992). The questions compass symptoms and signs relevant to disease, levels of physical activity, work, social interaction, sexual activity, and emotions. The MLHFQ total score range from 0 to 105 (no impairment to maximum impairment). Two other scores can be determined: the physical dimension (8 items, 0–40), and the emotional dimension (5 items, 0–25). Higher MLHFQ score mean worse QoL. Answers options ranges from 0 (none) to 5 (very much), where 0 represented no limitation and 105 represented maximal limitation.

Statistical analyses

Data normality was verified by Shapiro Wilk test. Not normally distributed variables were transformed into natural logarithm (weight, fat mass, free fat mass, 8FUG, MLHFQ total score, MLHFQ physical and MLHFQ emotional) for subsequent analysis and then transformed back to the original scale for the purpose of clarity. Data are expressed as mean \pm standard deviation. Categorical

data are reported as absolute values and percentages. Between gender comparisons were performed by independent *t*-test and chi-square test as appropriated. Pearson's correlation was used to analyse the relationship between physical fitness components (dynamic balance and mobility, upper body strength, CRF and BMI) to verify collinearity between variables ($r > 0.75$). Partial correlation (adjusted for age, gender and NYHA class) was used to assess the association between physical fitness components and dimensions of QoL. Multivariate linear regression analysis, with stepwise selection of variables was performed to determine the relationship between QoL dimensions with age, gender, NYHA functional class and physical fitness components, that were identified as potentials independent predictors of QoL. Statistical analysis was performed using the IBM SPSS 24 software (SPSS, USA), and the statistical significance was set at $p < 0.05$.

RESULTS

Patient's characteristics

The demographic and clinical sings of patients are presented in Table 1. Patients' mean age was 76 ± 6 years old, ranging from 59 to 85 years, and 71% (n=17) were female. Overall, mean weight was 72 ± 16 kg and % body fat was $36 \pm 7\%$. Hypertension was the most prevalent comorbidity (n=22, 92%), followed by dyslipidemia (n=17, 71%) and obesity (n=14, 58%). Regarding NYHA functional class, 79% (n=19) of all patients were classified as class II. The BNP average was 288.9 ± 191.5 pg/mL. Regarding cardiac function, mean ejection fraction was $60 \pm 6\%$, E/e' ratio was 12.2 ± 3.1 , and 23% (n=6) of patients had $E/e' > 15$. E/A ratio was 1.0 ± 0.52 . LAVI was 44.2 ± 11.7 mL/m², while 90% (n=22) of patients had LAVI > 34 mL/m², and LVMI was 231 ± 95 g/m². All patients had left ventricular hypertrophy. Comparisons between genders showed that male compared to female tended to be heavier (p=0.003), with more free fat mass (p<0.001) and exhibit higher waist circumference (p=0.04). Additionally, male compared to female (Table 1) had higher frequencies of being former smoker's (p=0.001) and history of chronic obstructive pulmonary disease (p=0.021) compared to female (Table 1).

Table 1: General patients characteristics (overall and by gender).

	All (n=24)	Women (n=17)	Men (n=7)
Sociodemographic characteristics			
Age (years)	76 ± 6.1	77 ± 6.2	74 ± 6
Female (n) (%)	17 (71%)	-	-
Anthropometrics			
Weight (kg)	72 ± 16	66 ± 10.2	$86 \pm 18.8^*$
Body mass index (kg/m ²)	31 ± 5	31 ± 5	32 ± 5
Fat mass (%)	36 ± 7	38 ± 5	33 ± 8
Free fat mass (kg)	45 ± 10	41 ± 5	$57 \pm 9^*$
Trunk fat (%)	$35\% \pm 6$	33 ± 5	37 ± 9
Waist circumference (cm)	101 ± 13	98 ± 10	$109 \pm 14^*$
Risk factors, n (%)			
Obesity (BMI ≥ 30 kg/m ²)	14 (58%)	9 (53%)	5 (72%)
Former smoker	4 (17%)	0	4 (57%)*
Hypertension	22 (92%)	15 (88%)	7 (100%)
Dyslipidemia	17 (71%)	11 (65%)	6 (86%)

Type 2 diabetes	2 (8%)	1 (6%)	1 (14%)
Pre-diabetic	9 (38%)	5 (29%)	4 (57%)
Atrial fibrillation	12 (50%)	8 (47%)	4 (57%)
Atrial fibrillation (paroxysmal)	4 (17%)	3 (18%)	1 (14%)
COPD	2 (8%)	0	2 (29%)*
Obstructive sleep apnea	6 (25%)	2 (12%)	4 (57%)
Clinical signs			
Resting HR (bpm)	72 ± 16	73 ± 16	73 ± 19
SBP (mmHg)	136 ± 19	134 ± 18	142 ± 22
DBP (mmHg)	70 ± 14	69 ± 15	74 ± 10
BNP (pg/mL)	289 ± 192	272 ± 171	331 ± 224
NYHA Functional class			
NYHA class I (%)	1 (4%)	0	1 (14%)
NYHA class II (%)	19 (79%)	13 (77%)	6 (86%)
NYHA class III (%)	4 (17%)	4 (24%)	0
Medication, n (%)			
ACE-i/ARB	17 (71%)	12 (71%)	5 (72%)
β-Blocker	20 (83%)	14 (82%)	6 (86%)
Loop diuretics	18 (75%)	11 (65%)	7 (100%)
Statin	16 (67%)	11 (65%)	5 (72%)
Digoxin	4 (17%)	3 (18%)	1 (14%)
MRAs	2 (8%)	1 (6%)	1 (14%)
Cardiac Function			
LVEF (%)	60 ± 6.3	61 ± 6	58 ± 6
E/e'	12.2 ± 3.1	12.5 ± 3.2	11.6 ± 2.9
E/A	1.0 ± 0.5	1.02 ± 0.5	0.97 ± 0.5
LVMI (gm/m ²)	231 ± 95	203 ± 61	279 ± 126
LAVI (mL/m ²)	44.2 ± 11.7	44.8 ± 12.5	42.5 ± 10.3

BMI: body mass index; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; NYHA: New York Heart Association; AF: atrial fibrillation; COPD: chronic obstructive pulmonary disease; ACEi/ARB: angiotensin-converting enzyme inhibitor and angiotensin receptor blocker; MRAs: mineralocorticoid receptor antagonists; EF: ejection fraction; E/e': ratio of early mitral transmitral flow velocity with early diastolic velocity of the mitral valve annulus; E/A: mitral ratio of peak early to late diastolic filing velocity; LVMI: left ventricle mass index; LAVI: left atrium volume index. Data are mean ± SD. *p<0.05.

Quality of life

Table 2 shows results from MLHFQ. The score of total MLHFQ scale was 26±24, whereas the physical and emotional MLHFQ subscales scores were 12±13, and 5±7, respectively. No significant differences were found between women and men (p>0.05).

Table 2: Patients results from total score, physical and emotional dimensions of QoL.

	Participants (n=24)	Women (n=17)	Men (n=7)
Quality of life			
MLHFQ total score	26 ± 24	30 ± 26	14 ± 12
MLHFQ physical	12 ± 13	15 ± 14	4 ± 4
MLHFQ emotional	5 ± 7	6 ± 7	3 ± 3

MLHFQ: Minnesota Living With Heart Failure Questionnaire. Data are mean ± SD.

Physical fitness

Physical fitness results are presented in Table 3. Overall, 6MWT distance, 8FUG and handgrip results were 10.9±3.6 seconds, 18.6±7.1kg, and 312±90 meters, respectively. Comparisons between genders showed that male compared to female had higher grip strength ($p<0.001$).

Table 3: Patient's results from physical fitness.

		All (n=24)	Women (n=17)	Men (n=7)
Physical Fitness dimensions				
Dynamic balance and mobility	8FUG (s)	10.8 ± 3.6	11 ± 3.5	10.2 ± 4
	Handgrip (kg)	18.8 ± 7	15.6 ± 4.4	26.3 ± 6.3*
CRF	6MWT			
	Distance (m)	312 ± 90	316 ± 91	301 ± 92
	VO ₂ (mL.min ⁻¹ .kg ⁻¹)	11.2 ± 2.2	11 ± 2.2	11.6 ± 2.2
	VCO ₂ (mL.min ⁻¹)	728 ± 183	684 ± 157	836 ± 209
	VE (L.min ⁻¹)	28.6 ± 7.1	27.6 ± 7.3	31.2 ± 6.3
	VE/VO ₂	36.8 ± 7.81	38.8 ± 8.3	32 ± 3.1*
	VE/VCO ₂	40 ± 5.69	40.7 ± 6.4	38 ± 2.5
	RER	0.92 ± 0.11	0.90 ± 0.12	0.85 ± 0.09
	HR (bpm)	103 ± 33	100 ± 23	113 ± 33
Body composition	BMI (kg/m ²)	31.1 ± 5.1	30.7 ± 5.4	32 ± 4.9

CRF: cardiorespiratory fitness; 8FUG: eight-foot-up-and-go test; 6MWT: six-minute walk test; BMI: body mass index; VO₂: oxygen uptake; VCO₂: carbon dioxide output; VE: minute ventilation; VE/VO₂: ventilatory equivalents for oxygen; VE/VCO₂: minute ventilation carbon dioxide output; RER: respiratory gas exchange ratio. Data are mean ± SD. * $p<0.05$.

Bivariate correlation between physical fitness components showed that the 8FUG test was inverse correlated with handgrip ($r=-0.47$; $p=0.01$) and 6MWT distance ($r=-0.81$; $p<0.001$) (Table 4).

Table 4: Bivariate correlation between physical fitness parameters.

	Dynamic balance and mobility	Upper body strength	Cardiorespiratory fitness	Body composition
	8FUG	Handgrip	6MWT	BMI
8FUG		-0.478*	-0.816**	-0.030
Handgrip	-0.478*		0.390	0.017
6MWT	-0.816**	0.390		-0.074
BMI	-0.030	0.017	-0.074	

8FUG: eight-foot-up-and-go test; 6MWT: six-minute walk test; BMI: body mass index. * $p<0.05$. ** $p>0.01$.

Association between physical fitness and quality of life

Partial correlation between dimensions of QoL and physical fitness components are presented in Table 5. Better MLHFQ total score was directly correlated with 8FUG ($r=0.563$; $p=0.008$) and inversely correlated with 6MWT ($r=-0.539$; $p=0.012$). Regarding to MLHFQ physical, it was directly correlated with 8FUG ($r=0.529$; $p=0.014$) and inversely correlated with 6MWT ($r=-0.478$; $p=0.028$). Finally, MLHFQ emotional was directly correlated with 8FUG ($r=0.597$; $p=0.004$).

Table 5: Partial correlation between dimensions of quality of life with physical fitness.

	Dynamic balance and mobility	Upper body strength	Cardio-respiratory fitness	Body composition
MLHFQ total	0.563*	-0.118	-0.539*	0.208
MLHFQ physical	0.529*	-0.261	-0.478*	0.260
MLHFQ emotional	0.597*	-0.023	-0.394	0.199

Adjusted for age, gender and NYHA functional class. 8FUG: eight-foot-up-and-go test; 6MWT: six-minute walk test; BMI: body mass index; MLHFQ: Minnesota Living with Heart Failure Questionnaire. * $p<0.05$.

Table 6 shows multivariate regression analysis for dimensions of QoL. All models were adjusted to age, gender and NYHA functional class as potential confounders. For MLHFQ total score, the 8FUG was the only parameter from physical fitness that remained an independent predictor ($\beta=0.651$; $p=0.001$) that explaining 42.4% of MLHFQ total score variance. The CRF was not an independent predictor of MLHFQ total score ($p>0.05$). Similarly, for MLHFQ physical dimension, the 8FUG was the single physical fitness component that remained as independent predictor ($\beta=0.570$; $p=0.04$) that explaining 32.4% of MLHFQ physical variance. The CRF was not an independent predictor of MLHFQ physical ($p>0.05$). Finally, for MLHFQ emotional, the 8FUG was the single physical fitness component that remained as independent predictor ($\beta=0.611$; $p=0.002$), explaining 37.3% of MLHFQ emotional variance.

Table 6: Stepwise regression analysis assessing which physical fitness components were independently associated with specific dimension of quality of life.

	β	B	R^2	p
MLHFQ total				
Ln 8FUG	0.651	5.015	0.424	0.001
MLHFQ physical				
Ln 8FUG	0.570	3.788	0.324	0.040
MLHFQ emotional				
Ln 8FUG	0.611	3.003	0.373	0.002

Ln 8FUG: natural logarithm of eight-foot-up-and-go test; MLHFQ: Minnesota Living with Heart Failure Questionnaire; β : standardized regression coefficient; B: non-standardized regression coefficient; R^2 : adjusted coefficient of determination.

DISCUSSION

The data provided by our study indicates that physical fitness is positively correlated with QoL in HFpEF patients. In addition, dynamic balance and mobility was the unique physical fitness component that was independently associated with QoL total score, and physical and emotional dimensions. These findings suggest that this specific component of physical fitness outperforms CRF in capturing HFpEF patients' QoL. In addition, it highlights the need to study interventions targeting specifically these fitness components to enhance QoL gains.

Despite the high prevalence and poor prognostic of HFpEF, evidence-based therapies to convincingly reduce morbidity or mortality remains to be developed (Holland et al., 2011). These patients are often characterized by poor quality of life and current treatment guidelines highlight the importance of aiming to improve patients well-being (Ponikowski et al., 2016). Physical fitness is multicomponent construct (Rikli & Jones, 2013) and several studies shows it is a major determinant of QoL in HFpEF (Kitzman et al., 2010; Nolte et al., 2015). Our results corroborate this finding, once we observed that QoL total score strongly correlated with physical fitness (e.g. dynamic balance and mobility, and CRF) in HFpEF patients.

Because physical fitness might influence QoL, strategies targeting physical fitness might potentially improve QoL, independently of further health benefits (Pandey et al., 2015). A recent meta-analysis showed that the combination of endurance exercise training together with cardiovascular drugs provide a clinically relevant improvement in both exercise capacity and QoL in HFpEF patients (Fukuta et al., 2016). However, physical fitness and QoL are multicomponent and multidimensional, respectively, and it is crucial to ascertain which dimension/component is better related to each other to maximize possible QoL improvements.

Previous studies have shown that CRF is mainly associated with the physical dimension, but not necessarily with the total score or emotional dimension of QoL (Edelmann et al., 2011a; Kitman et al., 2010). We observed that CRF (assessed by 6MWT) and dynamic balance and mobility (assessed by

8FUG) were both associated with physical dimensions of QoL. Moreover, dynamic balance and mobility was the only physical fitness component associated with emotional dimension of QoL, while upper body strength (assessed by handgrip) and body composition were not associated with any dimension. In addition, multivariate analysis revealed that the dynamic balance and mobility was the only physical fitness component independently associated with all dimensions of QoL, explaining 42% of variance in the total score QoL, 32% of physical dimension and 37% of emotional dimension of QoL. Thus, of all the components of physical fitness, dynamic balance and mobility seems to be the one that better capture QoL in HFpEF patients.

Collectively, our data suggest that improving the specific physical fitness component of dynamic balance and mobility, will eventually proportionate the greatest improvement in QoL. 8FUG reflects the specific demands of activities such as standing up from a seated position, walking short distances, turning, stopping and sitting down (Wall et al., 2000). This might be explained by the wide range of physical abilities, including lower body strength, dynamic balance, walking ability, agility and gait speed (Rikli & Jones, 2013) involved in the 8FUG. These abilities are also required in the normal daily tasks of an independent and autonomous life, especially in older people (Mlinac & Feng, 2016). Future studies (e.g. longitudinal training programs) should assess if an exercise training program focused on enhancing motor abilities (e.g. dynamic balance and mobility) will improve the physical and emotional components of QoL in HFpEF in comparison to current standard ones.

STUDY LIMITATIONS

The small sample size and the cross-sectional design of our study limits the generalization of our results. Despite that, our sample gathers the usual clinical features of HFpEF population reported in large studies (Ponikowski et al., 2016) with higher prevalence of elderly women and higher prevalence of comorbidities. Further prospective cohort studies with a larger sample size are needed to strengthen or refute our conclusions that dynamic balance and mobility is more discriminative to capturing HFpEF patients' QoL.

CONCLUSION

Overall, our findings indicate that both CRF and dynamic balance and mobility are directly associated with total score and physical dimensions of QoL in patients with HFpEF, but only dynamic balance and mobility was concomitantly associated with the emotional dimension. Multivariate analyses revealed that dynamic balance and mobility outperforms CRF in capturing HFpEF patients' QoL.

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STUDY II

COMPARISON OF QUESTIONNAIRE AND ACCELEROMETER-BASED ASSESSMENTS OF PHYSICAL ACTIVITY IN PATIENTS WITH HEART FAILURE WITH PRESERVED EJECTION FRACTION: CLINICAL AND PROGNOSTIC IMPLICATIONS

ABSTRACT

Rational: Heart failure with preserved ejection fraction (HFpEF) accounts for approximately 50% of heart failure cases and is associated with poor outcome. Higher physical activity (PA) levels are associated with greater quality of life and lower risk of hospitalization and mortality.

Purpose: The study aims: i) to compare daily PA levels and sedentary time evaluated by the International Physical Activity Questionnaire (IPAQ) and by a triaxial accelerometer in HFpEF patients; ii) to describe daily PA patterns and sedentary time in HFpEF patients based in objective measurements; and iii) to observe the association between prognostic indicators and PA, and sedentary time measurements.

Methods: This is a cross-sectional study with 24 stable and well-medicated HFpEF patients. Physical activity was assessed through the IPAQ short version and triaxial accelerometry (ActiGraph GTX3). Time spent in moderate-to-vigorous PA (MVPA) from IPAQ was computed as self-reported walking and MVPA. MVPA from accelerometry was set as ≥ 2752 counts/minute. Prognostic indicators were: distance on the 6-minute-walking test (6MWT), oxygen consumption (VO_2) during the test, quality of life, brain natriuretic peptide plasma level, and E/e' ratio.

Results: Sample mean age was 76 ± 4 years. Compared to accelerometry, IPAQ underestimated sedentary time (253 ± 156 vs. 392 ± 104 min/day, $p=0.001$) and overestimated MVPA (44 ± 56 vs. 19.3 ± 26 min/day, $p<0.001$). Using IPAQ, 59% of patients met international PA by international guidelines, while using accelerometer-derived data, 27% of patients actually met the guidelines, decreasing to zero when considering the number of 10min-MVPA bouts. On average, accelerometer-derived data showed that HFpEF patients spent 50% (392 ± 104 min/day) of their waking time in sedentary behaviours and 2.5% (19.3 ± 26 min/day) in MVPA. Of measured surrogate prognostic markers, functional capacity (6MWT, $r=0.652$, $p=0.04$; VO_2 , $r=0.512$, $p=0.02$) and quality of life ($r=-0.490$, $p=0.04$) were correlated with MVPA.

Conclusion: The IPAQ short version underestimated sedentary time and overestimated MVPA in patients with HFpEF. Using accelerometer-derived data, HFpEF patients spent only a minority of their time involved in MVPA, which was the only PA pattern positively associated with prognostic indicators. Our results highlight the importance of replacing some time spent in sedentary activities by MVPA in HFpEF throughout the day.

Key-words: physical activity, IPAQ, accelerometer, HFpEF, prognostic indicators.

INTRODUCTION

Epidemiologic evidence indicates that heart failure (HF) prevalence has been increasing in the last decade, with heart failure with preserved ejection fraction (HFpEF) accounting up to half of HF population (Oktay et al., 2013). The growing prevalence of HFpEF is attributed to life-expectancy expansion, better diagnostic awareness therapy (Glezeva & Baugh, 2014), and increasing prevalence of major risk factors (e.g. obesity, diabetes, hypertension and atrial fibrillation) (Ather et al., 2012). Consequently, it is expected that HFpEF will become the most common cause of hospitalization in older adults over the coming years (Nanayakkara et al., 2018). Since HFpEF continues to be resistant to current therapies, primary prevention strategies might be useful to control its growing burden at the population level (Pandey et al., 2017a). Therefore, in addition to understanding the natural history and pathophysiology of HFpEF, it will be important to identify modifiable risk factors that can be targeted.

Lifestyle risk factors such as low physical activity (PA) and high sedentary time are recognized as primary factors for most chronic diseases, including HF (Booth et al., 2012). While it remains to be clarified which HF phenotypes is most affected, recent observations have more strongly implicated low PA in the development of HFpEF (Pandey et al., 2017b). An inverse relationship between leisure-time PA and HF risk was recently demonstrate, where lower levels of PA were associated with higher risk of HFpEF, but not HF with reduced ejection fraction (Pandey et al., 2017). In addition, evidence shows that a sedentary lifestyle (e.g. prolonged bed rest) is associated with many underlying cardiac and skeletal muscle abnormalities often present in HFpEF (Dorfman et al., 2008; Irimia et al., 2017). For those with established HFpEF, lower levels PA have been associated with poor quality of life (QoL) and worse clinical outcomes such as functional class (NYHA) (Snipelisky et al., 2017), hospitalisation rate, and mortality (Hegde et al., 2017). Overall, these observational studies suggest that PA and/or sedentary time are important treatment targets to improve clinical outcomes in HFpEF.

Regardless the population, daily PA assessment is a challenging task. There are different methods (e.g. self-report methods such as questionnaires or

objective methods such as accelerometry), and each one has its own limitations and strengths. Despite of it, accelerometry seems to be superior than questionnaires in terms of accuracy, reliability, representatively of daily activities and related energy expenditure (Skender et al., 2016). Additionally, in clinical settings (e.g. diagnosis, prognosis, or prescription of therapeutic interventions) accelerometry might represent a challenge compared to questionnaires, which are better in terms of costs and practicality. However, correlation between self-report and objective PA measurements in the large majority of studies is poor (Lee et al., 2011). Additionally, there are no data comparing objective PA measurements (e.g. accelerometers) and questionnaire in HFpEF patients. Therefore, this study aimed: i) to determine the validity of the International Physical Activity Questionnaire (IPAQ) against objective measures from triaxial accelerometry in HFpEF patients; ii) to describe daily PA patterns and sedentary time in HFpEF patients based in objective measurements, and iii) to observe the association between prognostic indicators and PA and sedentary time measurements.

MATERIAL AND METHODS

Study design, recruitment and participants

This is a cross-sectional study conducted in a Portuguese public hospital (*Centro Hospitalar do Porto - Hospital de Santo Antonio*, Porto). Inclusion criteria was diagnosis of HFpEF according to the European Society of Cardiology guidelines (Ponikowski et al., 2016). Patients were excluded if they presented with unstable angina, acute coronary syndrome as primary diagnosis, symptomatic severe aortic stenosis, acute pulmonary embolus, acute myocarditis, uncompensated heart failure, uncontrolled hypertension, complex ventricular arrhythmias, severe renal dysfunction, severe chronic obstructive pulmonary disease, medical or orthopaedic conditions that precluded independent ambulation and exercise testing.

Patients who potentially were eligible to participate in the study were identified from the clinical files of the hospital's cardiology department. A total of 30 patients were invited through phone calls by a cardiologist. From those, 24 patients (17 female and 7 male) accepted to take part in the study, but only 22 completed data acquisition. The study was approved by the Ethics Committee of *Centro Hospitalar do Porto - Hospital de Santo Antonio* (N/S: 2015.125). All procedures were conducted according to the declaration of Helsinki, and participants signed informed consent to participate.

Data were collected from November 2016 to September 2017 in a single day in the hospital.

Data collection

Blood pressure

A trained researcher performed blood pressure measurements after 10 minutes resting in seated position. Blood pressure was assessed (Colin, BP 8 800; Critikron, Inc., USA) in the left arm. Systolic blood pressure (SBP) and Diastolic blood pressure (DBP) were computed as the average of 3 readings. Additional measurements were performed when differences between readings exceeded 5 mmHg (Mancia et al., 2014).

Blood collection and biochemical determinations

Peripheral venous blood (15 mL) was collected to EDTA tube. The EDTA tubes were immediately placed on ice and allowed to clot for 30 minutes before centrifugation for 15 minutes at 1000xg. The plasma was aliquoted and stored at -80°C for biochemical analysis. Brain Natriuretic Peptide (BNP) was quantified in a certificated laboratory by chemiluminescent microparticle immunoassay (ARCHITECT BNP).

Anthropometric Measures

Body height (cm) was measured standing upright against a stadiometer (Holtain Ltd., Crymmych, UK) (Lohman, 1988). Patients were bare or stocking feet. Weight (kg), body mass index (BMI; kg/m²), fat mass (%) and free fat mass (kg) were measured with patients lightly dressed, using an electronic segmental body composition analyser (Tanita, BC-418, Tokyo, Japan). Waist circumference (cm) was measured at the midpoint between the lowest rib and the iliac crest at the end of normal expiration (Riley et al., 2016). Obesity was determined as BMI equal or higher than 30 kg/m² (WHO, 2000).

Self-Reported Physical Activity

Self-reported physical activity was assessed with the short version of the International Physical Activity Questionnaire (IPAQ-SF) (Craig et al., 2003), through personal interview. The IPAQ contains seven items to estimate PA frequency and duration during the previous seven days. Additional questions on sedentariness were also asked. The IPAQ focus on vigorous and moderate physical activity intensities and walking periods lasting at least 10 minutes on week days. Additionally, the questionnaire asked about “sitting time” on week and weekend days. Frequency of activity was measured in days, and duration in hours and minutes. Total weekly PA was estimated using the instrument's scoring protocol by weighting time spent in each activity intensity with its estimated metabolic equivalent (MET) energy expenditure (Ipaq Research Committee, 2005). IPAQ scoring protocol assigns the values of 3.3 METs to walking, 4.0

METs to moderate, and 8.0 METs vigorous activity (Ipaq Research Committee, 2005). Meeting physical activity guidelines was defined as accumulating ≥ 150 minutes per week of moderate-intensity physical activity (MVPA) (≥ 3 METs) (Haskell et al., 2007). Thus, to estimate MVPA data, we merged the reported activities ≥ 3 METs (walking + moderate + vigorous PA).

Accelerometer-assessed physical activity

Daily PA was measured using a triaxial accelerometer (Actigraph GT3X, Pensacola, FL, USA). The triaxial activity monitor measures acceleration in three individual orthogonal planes (vertical, antero-posterior and medio-lateral) and provides activity counts as a composite vector magnitude (VM) of these three axes (Sasaki et al., 2011). The VM is the square root of quadrate of the three separate dimensional axes $[(x^2+y^2+z^2)^{1/2}]$ (Crouter et al., 2012). Participants were instructed to wear the accelerometer over the right hip for eight consecutive days, except while sleeping, bathing and water-based activities. The accelerometer was programmed to record triaxial data at a frequency of 30 Hz and 1 second length epochs. ActiLife software (Actigraph, Florida, USA, version 6.9) was used to process the accelerometer data. Data were downloaded and integrated into 60-second epochs. Non-wear time was defined as 90 consecutive minutes of zero counts, with an allowance of 2-minutes of nonzero counts provided there were 30-minute consecutive zero count windows up and downstream (Choi et al., 2011). Non-wear time was excluded from the analysis. Patients with valid data were those having a minimum of 4 days with at least 10 hours/day of wear-time. The output of the Actigraph was given in counts per minute (CPM) derived from VM. The average minutes/day spent at different categories of PA intensity was determined according to cut point that relate PA to counts/min: sedentary time (< 200 counts/min) (Aguilar-Farias et al., 2014), light PA (LPA) (200-2751 counts/min) and moderate and vigorous PA (MVPA) (> 2752 counts/min) (Santos-Lozano et al., 2013). Meeting international PA guidelines was defined as ≥ 150 minutes/week of MVPA from accelerometry. This definition was applied considering: i) only MVPA that occurred in bouts of ≥ 10 minutes' long (episodes of continuous MVPA lasting for at least 10 minutes) as specified by the guidelines

(Haskell et al., 2007), and ii) without bout length restriction (e.g. considering all MVPA including MVPA occurring in bouts shorter than 10 minutes' duration).

Functional classification

Patients were classified by the physician into subgroups based on their symptoms using the New York Heart Association (NYHA) functional class. Patients symptoms is based on how much they are limited during physical activity (class I to IV) (Dolgin, 1994).

Echocardiography Evaluation

Supine transthoracic echocardiography was performed using a cardiovascular ultrasound Vivid E95 ® (GE Healthcare). All quantitative echocardiographic measurements were performed by a single reader blinded to the results of the other evaluations, using a computerized off-line analysis station. Peak early diastolic tissue velocity was measured at the septal and lateral mitral annulus. Mitral inflow velocity was assessed by pulsed wave Doppler from the apical 4-chamber view, positioning the sample volume at the tip of the mitral leaflets. E/e' ratio was calculated as E wave divided by e' velocities. LV mass was estimated from LV linear dimensions and indexed to body surface area as recommended by ESC guidelines (Lang et al., 2015). LV hypertrophy was defined as LV mass indexed to body surface area (LV mass index) >115 g/m² in men or >95 g/m² in women. LV volumes were estimated by the modified Simpson method using the apical 4- and 2-chamber views, and LVEF was derived from volumes in the standard manner. LA volume was estimated by the method of disks using apical 4- and 2-chamber views at an end-systolic frame preceding mitral valve opening and was indexed to body surface area to derive LA volume index.

Cardiorespiratory fitness with pulmonary gas exchange analysis

Cardiorespiratory fitness was assessed by the 6-minute walk test (6MWT) in a 25-m-long unobstructed corridor. Participants were instructed to walk the maximal distance in 6 minutes time. Resting stops were allowed when patients

feel to be necessary. The 6MWT was performed wearing a portable gas analyser (K4b2, Cosmed, Rome, Italy). Oxygen uptake (VO_2 ; $\text{mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) was measured directly and continuously. Respiratory samplings were collected in a breath-by-breath, and then, data was averaged over 5-s intervals. Data was calculated as the averaged of measures taken in test total duration (6 minutes).

Health-related quality of life

Health-related QoL was performed by interview through the Minnesota Living With Heart Failure Questionnaire (MLWHFQ). The MLHFQ encompasses 21 questions, whose purpose is to determine how disease affects the physical, psychological and socioeconomic conditions of the patients during the previous month (Rector & Cohn, 1992). The questions compass symptoms and signs relevant to disease, levels of PA, work, social interaction, sexual activity, and emotions. The MLHFQ total score range from 0 to 105 (no impairment to maximum impairment). Two other scores can be determined: the physical dimension (8 items, 0–40), and the emotional dimension (5 items, 0–25). Higher MLHFQ score mean worse QoL. Answers options ranges from 0 (none) to 5 (very much), where 0 represented no limitation and 105 represented maximal limitation.

Statistical analyses

Statistical analysis was performed using the IBM SPSS 24 software (SPSS, USA). Normal data distribution was examined by the Shapiro Wilk test. Non-normal data was transformed with the square root (IPAQ-MVPA, IPAQ_METs/day, accelerometer-MVPA, accelerometer-10min-bouts-MVPA, MLHFQ total score, MLHFQ physical and MLHFQ emotional) or its natural logarithm (weight, fat mass, free fat mass, accelerometer total hours and accelerometer total days), for subsequent analysis and then transformed back to the original scale for the purpose of clarity. Categorical data are reported as absolute values and percentages. Between gender and age comparisons were performed by independent *t*-test and chi-square test as appropriated. Cut point to age analyses was defined by median, and it was set as 76 years old. Siting

time and walk+moderate+vigorous PA (MVPA) from IPAQ were compared with sedentary time, total MVPA and 10 min-bouts of MVPA derived from GT3X accelerometer, using paired t-test. Partial correlation was used to analyse the relationship between the variables derived from the two methods adjusted by gender and age. The strength and limits of agreement between the two methods was assessed using the Bland–Altman technique (Bland & Altman, 1986). Statistical significance was established for $p < 0.05$.

RESULTS

Patients' characteristics

Demographic and clinical characteristics of the participants are depicted in Table 1. On average, participants were 76 ± 4 years old (range 59-85 years), and 73% (n=16) were female. For the whole sample, the mean weight was 71 ± 16 kg and the % body fat was $36 \pm 6.6\%$. Hypertension was the most prevalent risk factor (n=20, 91%), followed by dyslipidaemia (n=15, 68%) and obesity (n=12, 55%). The majority of participants (n=17, 77%) were classified as NYHA class II. The BNP average was 272 ± 191.56 g/mL. Regarding to cardiac function, mean ejection fraction was $59.4 \pm 6.3\%$, the E/e' ratio was 12.2 ± 3.1 and 32% of patients had $E/e' > 15$. E/A ratio was 1.0 ± 0.5 , LAVI was 43 ± 12 mL/m² and LVMI 222 ± 96 gm/m². All patients had left ventricular hypertrophy, and 95% of patients had LAVI > 34 mL/m². Regarding cardiorespiratory fitness, the mean distance walked in the 6MWT was 312 ± 90 meters. The average of VO₂ during the test was 11.2 ± 2.3 mL.min.kg⁻¹. The total score of MLWHFQ was 25.2 ± 24.1 points.

Between genders comparisons showed that male compared to female tended to be heavier (p=0.004) and to have more free fat mass (p<0.001). Additionally, compared to female, male had higher frequencies of former smokers (p=0.002) as well as higher frequencies of chronic obstructive pulmonary disease (p=0.015) (Table 1).

Table1: Patients characteristics.

	All (n=22)	Women (n=16)	Men (n=6)
Body composition			
Weight	71 ± 16	65 ± 10	$86 \pm 21^*$
BMI (kg/m ²)	30.7 ± 5	30 ± 5	32 ± 5
Fat mass (%)	36 ± 7	37 ± 5	32 ± 8
Free fat mass (kg)	45 ± 10	41 ± 5	$58 \pm 10^*$
Trunk fat (%)	34 ± 6	38 ± 5	33 ± 8
Waist circumference (cm)	100 ± 13	97 ± 11	108 ± 16
Risk factors, n (%)			
Obesity (%)	12 (55%)	8 (50%)	4 (67%)
Former smoker (%)	3 (14%)	0 (0%)	3 (50%)*
Hypertension (%)	20 (91%)	14 (88%)	6 (100%)
Dyslipidemia (%)	15 (68%)	10 (63%)	5 (83%)
Type 2 diabetes (%)	2 (9%)	1 (6%)	1 (17%)

Pre-diabetic (%)	8 (36%)	5 (31%)	3 (50%)
AF (%)	11 (50%)	8 (50%)	3 (50%)
AF (paroxysmal) (%)	4 (18%)	3 (19%)	1 (17%)
COPD (%)	2 (9%)	0 (0%)	2 (33%)*
Obstructive Sleep Apnea (%)	5 (23%)	2 (13%)	3 (50%)
Clinical signs			
Resting HR (bpm)	73 ± 17	72 ± 16	74 ± 20
SBP (mmHg)	135 ± 18	134 ± 18	138 ± 21
DBP (mmHg)	70 ± 14	69 ± 16	72 ± 7
BNP (pg/mL)	272 ± 180	273 ± 176	273 ± 206
NYHA Functional Classification			
NYHA class I (%)	1 (5%)	0 (0%)	1 (17%)
NYHA class II (%)	17 (77%)	12 (75%)	5 (83%)
NYHA class III (%)	4 (18%)	4 (25%)	0 (0%)
Medication			
ACE-i/ARB (%)	16 (73%)	11 (69%)	5 (83%)
β-Blockers (%)	18 (82%)	13 (81%)	5 (84%)
Loop diuretics (%)	16 (73%)	10 (63%)	6 (100%)
Statins (%)	14 (64%)	10 (63%)	4 (67%)
Digoxin (%)	3 (14%)	3 (19%)	0 (0%)
MRAs (%)	2 (9%)	1 (6%)	1 (17%)
Cardiac Function			
LVEF (%)	59.4 ± 6.3	60 ± 6	57 ± 6
E/e'	12.2 ± 3.1	12.9 ± 3.1	11 ± 2.6
E/A	1.0 ± 0.5	1.02 ± 0.5	0.97 ± 0.5
LVMI (gm/m ²)	222 ± 96	193 ± 51	277 ± 138
LAVI (mL/m ²)	43 ± 12	44.1 ± 13	38.7 ± 7
Physical fitness			
6MWT (m)	313 ± 91	312 ± 92	315 ± 95
VO ₂ (ml/min/kg)	11.2 ± 2.3	11 ± 2.3	11.7 ± 2.4
Quality of life Questioner			
MLHFQ total (points)	24 ± 24	29 ± 26	11 ± 10
MLHFQ physical (points)	12 ± 13	15 ± 14	4 ± 4
MLHFQ emotional (points)	5 ± 7	6 ± 7	3 ± 3

BMI: Body mass index; AF: atrial fibrillation; COPD: chronic obstructive pulmonary disease; HR: heart rate; SPB: systolic blood pressure; DBP: diastolic blood pressure; BNP: brain natriuretic peptide; NYHA: New York Heart Association; ACEi/ARB: angiotensin-converting enzyme inhibitor and an angiotensin receptor blocker; MRAs: mineralocorticoid receptor antagonists; EF: ejection fraction; E/e': ratio of early mitral transmitral flow velocity with early diastolic velocity of the mitral valve annulus; E/A: mitral ratio of peak early to late diastolic filling velocity; LVMI: left ventricle mass index; LAVI: left atrium volume index; 6MWT: six-minute walk distance test; VO₂: relative oxygen uptake; MLHFQ: Minnesota Living With Heart Failure Questionnaire. Data are mean ± SD * p<0.05.

Differences between self-reported and objective measures of PA

The results of self-reported and objective measures of PA are described in Table 2. Accelerometer was used an average of 6.4±0.9 days, with a mean daily wear time of 790 minutes (13.2±1 hours/day). Compared to female, male wore the accelerometer during less time (741±86 vs 808±46 min/day, p=0.02).

Mean total activity volume from IPAQ was 152 ± 183 MET/min/day, with older patients compared to younger, reporting lower activity volume (232 ± 71 vs 72 ± 114 MET/min/day, respectively; $p=0.03$). From accelerometry, mean total activity volume was 550 ± 239 CPM.

Mean sedentary time was significantly lower in self-reported measurement (253 ± 156 min.day⁻¹) compared to accelerometry (392 ± 104 min.day⁻¹, $p=0.001$). No significant differences were found regarding to gender and age ($p>0.05$). Considering MVPA, self-reported daily time compared to accelerometer was higher (44 ± 56 vs. 19.3 ± 26 min/day, respectively; $p<0.001$). Applying the ≥ 10 min-bouts at MVPA criterion, the discrepancy between measurements was even higher (IPAQ= 44 ± 56 min/day; Accelerometry= 1.1 ± 2.4 min/day, $p<0.001$). From accelerometry data, it was possible to verify that only 5 patients accomplished with at least one bout of MVPA per week. Gender comparison showed that female compared to male, spent less time in 10 min-bouts of MVPA per day (0.2 ± 0.8 vs 3.4 ± 7 , respectively; $p=0.01$). Analysis by age showed that older patients spent significantly less time in MVPA (34 ± 31 vs 5 ± 3 , $p=0.002$) and did not perform 10 min-bouts of MVPA.

Regarding to LPA, comparisons are not possible because it is measured only via accelerometry. Mean time in LPA was 379 ± 128 min/day. Gender comparisons show that male compared to female spent less time in LPA (423 ± 111 vs 260 ± 91 , respectively; $p=0.005$), while no significant difference was found comparing Youngers and elders ($p>0.05$).

Table 2: Descriptive PA levels from IPAQ and accelerometer variables.

	All (n=22)	Women (n=16)	Men (n=6)	≤76 y (n=11)	>76 y (n=11)
IPAQ-S Questionnaire					
IPAQ Total Activity (MET.min.d ⁻¹)	152±183	149±204	159±123	232± 71	72±114*
IPAQ Sitting (min.d ⁻¹)	253±156	226±141	323±184	280±138	224±173
IPAQ MVPA (min.d ⁻¹)	44±56	43±62	46±35	66±64	21±34
Accelerometer					
Total wear (min.d ⁻¹)	790±65	808±46	741±86*	803±60	775±68
Total activity (counts.min ⁻¹)	550±239	574±214	489±313	641±271	459±170
Sedentary time (min.d ⁻¹)	392±104	370±98	450±103	386 ± 123	396±85
Light activity (min.d ⁻¹)	379±128	423±111	260±91*	383 ± 134	374±127
MVPA (min.d ⁻¹)	19.3±26	15±21	31±37	34 ± 31	5±3*
MVPA 10min-bouts (n)	0.07±0.14	0.02±0.07	0.1±0.2	0.13±0.18	0.00±0.00*
MVPA 10min-bouts (min.d ⁻¹)	1.1±2.4	0.2±0.8	3.4±7*	2.2±3	0.00±0.00*

Age group was divided by median. IPAQ: international physical activity questionnaire; MVPA: moderate to vigorous physical activity. Data are mean ± SD. *p<0.05.

Correlation and agreement between self-reported and objective measures of PA

To compare the validity and the accuracy of self-report and accelerometer-derived PA were analysed: the standard error of the estimate (SEE), the validity correlation, the systematic error and the 95% limits of agreement (Table 3). Validation coefficients (correlation between self-report and accelerometer measured) were not significant in all PA levels, even after adjustments for gender and age. A significant mean difference (systematic error; $p>0.05$) between self-reported and accelerometer-derived PA levels was detected in sedentary time and in 10-min-bouts-MVPA. By analysing the 95% limits of agreement, both PA levels presented a higher variation, ranging from -427.8 to 150 min/day for sedentary time, and from -65.2 to 150.5 min/day in 10-min-bouts-MVPA. The SEE was 96.7 min/day for sedentary time, 26.7 min/day for MVPA and 2.4 min/day for 10-min-bouts-MVPA (Table 3).

Table 3: Descriptive values for self-report physical activity levels.

	Self-Reported (Mean \pm SD)	Mean Difference (95% LOA)	<i>r</i> (<i>p</i>)	SEE
Sedentary time	253 \pm 156	-138.9 (-427.8; 150)*	0.368 (0.111)	96.7
MVPA	44 \pm 56	24.4 (-88.9; 137.8)	0.261 (0.266)	26.7
MVPA 10-min-bouts	44 \pm 56	42.6 (-65.2; 150.5)*	0.236 (0.317)	2.4

Mean Difference= self-reported - objective measured parameter (expressed in min/day); LOA = limits of agreement (mean difference \pm 1.96 SD); *r* = Pearson correlations between self-reported and measured parameters (validation coefficient); SEE = standard error of the estimate expressed in min.d⁻¹; **p*<0.05 for comparison between self-reported and measured parameters.

Separate Bland-Altman plots were build-up for sedentary time, total MVPA and 10min-bouts-MVPA (Figure 1). Both MVPA plots show a linear tendency, which is not acceptable for the agreement between the two methods. Analysis of 10min-bouts of MVPA revealed that at higher levels of PA, the difference between self-reported data and accelerometers data becomes greater. In these cases, the self-reported levels were greater than what was observed by the tri-axial accelerometer.

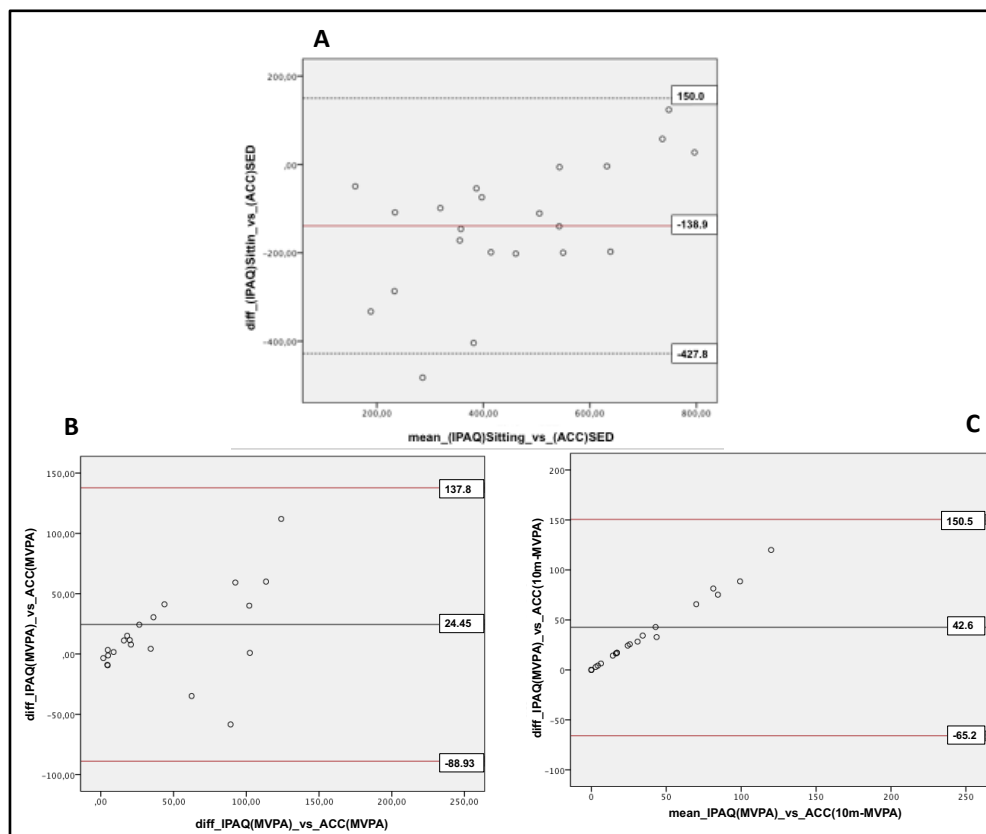


Figure 1: Bland-Altman plot of the mean bias and 95% limits of agreement for time spent in (A) sedentary time, (B) MVPA and (C) 10-min-bouts-MVPA. Black line indicates mean difference (systematic error); red lines indicate the 95% limits of agreement (mean \pm 1,96 SD). SD: standard deviation. MVPA: moderate-to-vigorous physical activity.

Associations between objective measures of PA with prognostic indicators

The association between objective measures of PA and prognostic indicators is showed in Table 4). Total activity (CPM) was significant associated with VO_2 ($r=0.498$, $p=0.04$). Furthermore, MVPA was positively correlated with VO_2 ($r=0.512$; $p=0.02$) and 6MWT ($r=0.652$; $p=0.008$), and inversely correlated with QoL ($r=-0.490$; $p=0.04$). In addition, 10min-bouts of MVPA was positively correlated with VO_2 ($r=0.559$; $p=0.04$) and inversely correlated with QoL ($r=-0.465$; $p=0.03$). No significant correlations were found between sedentary time or LPA with any prognostic indicators.

Table 4. Partial correlations between accelerometer measured sedentary time MVPA, MVPA 10min-bouts and prognosis values.

	VO_2	6MWT	QoL	BNP	E/e'
Total activity (CPM)	0.509*	0.271	-0.371	-0.097	-0.068
Sedentary time	-0.323	-0.012	-0.128	-0.025	0.203
Light activity	0.367	0.225	-0.211	0.318	0.183
MVPA	0.512*	0.652*	-0.490	-0.068	-0.133
MVPA 10min-bouts	0.459*	0.427	-0.559*	0.022	-0.016

CPM: counts per minute; MVPA: moderate to vigorous physical activity; VO_2 : oxygen uptake; 6MWT: six-minute walk distance test; QoL: quality of life; BNP: brain natriuretic peptide; E/e': ratio of early mitral transmitral flow velocity with early diastolic velocity of the mitral valve annulus.

* $p<0.05$.

DISCUSSION

The main findings of this study suggest that: i) in comparison to accelerometer, self-reported PA from IPAQ-SF underestimates sedentary time and overestimates time spent in MVPA in HFpEF patients, ii) HFpEF patients spent only a minority of their daily activity in MVPA, and iii) only MVPA measured from accelerometer was correlated with functional capacity and QoL.

Despite the evidence showing that questionnaires are valid and reliable in measuring PA, their correlation with objective measures (e.g. accelerometers) is far from satisfactory (Koolhaas et al., 2018; Lee et al., 2011; Prince et al., 2008; Skender et al., 2016), which limits PA-based decisions. In agreement with these observations, our data highlights for the first time the disparity of PA measurements obtained with IPAQ-SF in comparison to accelerometer-derived data in HFpEF patients, with the former overestimating MVPA and underestimating sedentary/sitting time. We also verified the absence of agreement between self-reported and accelerometer-derived sedentary time and MVPA. This means that measuring PA levels in the same person with these instruments leads to important variations in magnitude and concordance of the measured outcome.

According to self-reported PA, 59% of our patients would meet the international recommendations for MPVA (>150 min/week of MVPA). However, when we confronted with accelerometer-assessed data, this percentage dropped to 27% (without the 10min bout criteria) but decreased to zero when the 10min-bouts criteria was considered. A similar finding was found in the Women's Health Study, where 67% of women meet PA recommendations when considering results from self-reported methods, but when considering accelerometry (using 10min-bouts criteria), this percentage was 19% (Shiroma et al., 2015). The lack of correlation and agreement between methods may be related with different constructs measured by the two instruments. While the accelerometer measures the motion through acceleration of body mass, the IPAQ-SF questionnaire measures the time spent in specific behaviours (Troiano et al., 2014). Therefore, the use of objective measures of PA seems to have a particular importance to

avoid bias in populations with limited physical function and limited past knowledge and experience on regular PA (Prince et al., 2008).

Given the growing recognition of the impact of PA levels in the prognosis of HFpEF patients, a rigorous characterization of their patterns is crucial for prescribing tailored life-style changes. In our study, descriptive accelerometer data shows that HFpEF patients spent 50% of recorded time in sedentary behaviours, 47.5% at LPA and only 2.5% at MVPA (0.1% with 10min-bout criteria). Similar patterns were recently observed by Yavari and colleagues, where HFpEF patients reported to spend most of their waking time in sedentary behaviours while their daily activity was mainly comprised of LPA, and just a few minutes in MVPA (Yavari et al., 2017). Overall, these patterns of PA are similar to those described for adults older than 60 years, where sedentary time accounted for approximately for 60% of their waking time, LPA for 40% and 4% for MVPA (Spittaels et al., 2012). Although the patterns of time spent in each category are similar, our results show that the proportion of time spent in LPA was almost 10% higher, while sedentary time was 10% lower. While the fact that our patients were in optimal medical control may have accounted for that, differences in the type of accelerometers and cut-points also may have influenced the results. In fact, we used the GT3X triaxial accelerometer with the low frequency extension filter, which was shown to be more sensitive to capture slower movements, translating into decreased sedentary time and increase time in all PA intensities (Migueles et al., 2017). In addition, although specific cut-points for GT3X triaxial accelerometer have not been validated in HFpEF patients, we used cut-points for older adults validated with the GT3X, which we believe are more representative for our population.

With respect to prognosis, it was recently shown in the NEAT-HFpEF trial that accelerometer-derived total daily PA in HFpEF patients was associated with better 6MWT, NYHA functional class, QoL, and NT-proBNP levels (Snipelisky et al., 2017). Our study adds the novelty that only time spent in MVPA was significantly associated with important clinical outcomes as VO_2 , 6MWT and QoL, but not with E/e' or BNP levels. Thus, it seems that intensity is a requirement for significantly impacting the patient's prognosis. Corroborating this hypothesis, it

was recently shown a dose-response relationship between MVPA and risk of hospitalization or mortality in HFpEF patients (Hegde et al., 2017). In addition, it should be noted that only a minority of patients achieved the weekly recommendations of MVPA (without the 10min-bouts criteria). While any increase in the amount of PA may translate into some health benefits (Physical Activity Guidelines Advisory Committee Scientific Report, 2018), our data suggests that patients should be educated about the importance of reducing their sedentary time and engage in more MVPA for greater benefits.

STUDY LIMITATIONS

Some limitations should be acknowledged. The small sample size limits inferences regarding the agreement between PA measurements from IPAQ-SF questionnaire and PA measures derived from triaxial accelerometer. This report has a cross-sectional design, which limits the establishment of causal inferences regarding PA and its influence on prognostic indicators, and the sample size preclude the determination of predictive models. Moreover, the sample in this study does not reflect all stages of the disease course.

CONCLUSION

Our results suggest that IPAQ-SF, compared to objectively measured PA, underestimates sedentary time and over-estimates time spent in MVPA in patients with HFpEF, limiting its use to support accurate recommendations. Accelerometer-derived data shows that HFpEF patients spent only a minority of their time involved in MVPA, which was the only PA pattern positively associated with prognostic indicators, highlighting the importance of reducing sedentary time and performing MVPA throughout the day.

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STUDY III

CHRONIC EXERCISE TRAINING REDUCES LEFT VENTRICLE STIFFNESS
IN AN ANIMAL MODEL OF HEART FAILURE WITH PRESERVED EJECTION
FRACTION

ABSTRACT

Rational: In heart failure with preserved ejection fraction (HFpEF), microvascular endothelial inflammation is responsible for cardiomyocyte stiffness and interstitial fibrosis which leads to diastolic left ventricle (LV) dysfunction. Nonetheless, HFpEF optimal treatment remains widely undefined.

Purpose: To assess the impact of exercise training at the level of cardiac function and characterize underlying cellular and molecular changes.

Methods: Nine-week old ZSF1 obese rats (Ob n=20) were divided into two groups: sedentary (ObSED, n=10) and exercised (ObEX, n=10). At the 16th week of life, the ObEX group was submitted to treadmill exercise training for 4 weeks, 5 days per week, 60 min per day, at a speed of 20m/min. At the end of the protocol (20th week), all animals performed a maximal oxygen consumption (VO₂max) test, hemodynamic evaluation, and samples were collected for analysis. Plasma proteins were analysed by ELISA. Samples from LV were collated for: i) histological analysis (cross-sectional area (CSA), collagen content) and ii) passive tension analysis in skinned cardiomyocytes.

Results: Exercise training improved VO₂max (21 ± 2.5 vs 17 ± 1.8 mL.Kg⁻¹.min⁻¹, p=0.005). The end-diastolic pressure volume relationship had a trend to decrease in ObEX that, together with the decreased passive tension presented in skinned cardiomyocytes (p<0.05) and reduced collagen/muscle ratio (p<0.05), suggests a reduction in myocardial stiffness. No significant differences were observed between the groups in CSA. In addition, exercised animals showed reduced levels of several circulating biomarkers, such as markers of inflammation, endothelial dysfunction, oxidative stress, neurohumoral activation and extracellular matrix remodelling (p<0.05).

Conclusion: Chronic exercise training improved exercise capacity and attenuated LV diastolic function and in rats with HFpEF. These improvements were associated with decreased cardiomyocyte passive tension and extracellular matrix fibroses. Moreover, exercise training reduced circulating levels of inflammatory cytokines and markers of endothelial dysfunction, oxidative stress, neurohumoral activation and extracellular matrix remodelling.

Key-words: exercise training, LV stiffness, HFpEF, circulating biomarkers.

INTRODUCTION

Heart Failure (HF) with preserved ejection fraction (HFpEF) is a global clinical problem, accounting for more than 50% of all HF cases (Dunlay et al., 2017). Its prevalence has strikingly grown due to increasing prevalence of risk factors such as obesity, hypertension and diabetes in an ageing population (Dunlay et al., 2017). Heart Failure with preserved ejection fraction is responsible for frequent hospitalizations, high consumption of resources and poor prognosis, and no treatment has yet been shown to reduce morbidity or mortality (Owan et al., 2006; Ponikowski et al., 2016). Ventricular diastolic dysfunction is a central feature of HFpEF, caused by impaired relaxation and increased diastolic stiffness (Redfield, 2016). This is mediated, at least in part, by extracellular matrix remodeling (van Heerebeek et al., 2012) and changes in the expression and phosphorylation of cytoskeletal proteins (Hamdani et al., 2013).

Exercise training is emerging as an important ally for the clinical management of health-related outcomes in HFpEF patients. Different meta-analysis of randomized clinical trials shows that exercise training, in comparison to usual care, improves functional capacity, cardiorespiratory fitness and quality of life (Fukuta et al., 2016; Pandey et al., 2015). It has been proposed that non-cardiac peripheral adaptations are the major contributors (Haykowsky & Kitzman, 2014). However, some clinical (Dieberg et al., 2015; Pearson et al., 2017) and pre-clinical studies (Hidalgo et al., 2014; Marshall et al., 2013) support the hypothesis that exercise training may also improve diastolic function. Whether this will translate into a greater peak cardiac output remains unknown. Understanding the pathways that mediate these cardiac benefits could yield novel therapeutic approaches based on agents that mimic or potentiate the physiological benefits of exercise. In this sense, it was already shown that chronic free-wheel running improved cardiac stiffness in a genetic mouse model of HFpEF, an effect that was mediated by the reduction on titin-based passive stiffness in a phosphorylation-dependent manner (Hidalgo et al., 2014; Slater et al., 2017). Using the Yucatan miniature swine model of HFpEF, Marshall and collaborators also showed that treadmill exercise training attenuated diastolic impairment by reducing extracellular matrix (ECM) fibrosis (Marshall et al., 2013).

Despite these molecular insights, it is important to highlight that none of these animal models fully mimic the spectrum of changes found in humans with HFpEF (Ponikowski et al., 2016). Recently, our group contributed to surpass these limitations by validating the ZSF1 obese animal model for HFpEF (Hamdani et al., 2013). The ZSF1 obese animals exhibit several features of human HFpEF such as the presence of comorbidities, exercise intolerance, and diastolic dysfunction with preserved systolic function (Hamdani et al., 2013, Leite et al., 2015), placing this model one step ahead towards clinical translation (Lourenco et al., 2018). Therefore, by using this animal model of HFpEF, we aimed to assess the impact of exercise training at the level of cardiac function and characterize underlying cellular and molecular changes.

METHODS

Animal model

The animal protocol was approved by the ethics committee of Faculty of Medicine of Porto and was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication no. 85-23, revised 1996).

Nine-week-old rats ZSF1 obese (Zucker diabetic fatty/Spontaneously hypertensive heart failure F1 hybrid rats) (ZSF1 Ob, n=17) were obtained from Charles River (Barcelona, Spain). Animals were housed in groups of 2 animals per cage, maintained on a 12-hour light-dark cycle, at a room temperature of 22°C, fed with Purina Diet (#5008) and water *ad libitum*. Rats were allowed 1 week of adaptation to laboratory conditions before experiments. At 16th week of life, the ZSF1 Ob rats were randomly divided into two groups, obese sedentary (ObSED, n=7; normal activity at the cage) and obese exercised (ObEx, n=10; aerobic exercise training). Exercise training protocol consisted of running on the treadmill (76-0896, PanLab Harvard Apparatus) 5 days per week, 1 hour per day, at a speed of 20m/min, for 4 weeks. The sedentary group was submitted to a stationary treadmill in order to minimize environmental confounders among the animals not subjected to the exercise protocol. The protocol was interrupted 48 hours before terminal evaluation and sample collection to avoid confounding with acute effects of the last training session.

Peak effort testing with oxygen consumption determination

At 20th week, animals underwent an exercise tolerance test with oxygen consumption (VO_2) measurement (LE8700C, OxyletPro System, PanLab Harvard Apparatus). Initially, animals were adapted to treadmill at 9m/min for 3 minutes, followed by a rapid effort escalation to assess peak oxygen consumption ($\text{VO}_{2\text{peak}}$). Velocity was changed to 18m/min and then stepped up 3m/min at 1min intervals. Treadmill was stopped whenever animals were unable/unwilling to maintain pace, as defined by incapability to come off the back of the treadmill lane for >3 seconds. Gas concentrations (O_2 and CO_2), speed and covered

distance were continuously recorded. Data analysis was performed with the aid of software (Metabolism V2.2.01, Panlab Harvard Apparatus®).

Hemodynamic evaluation

Forty-eight hours after exercise testing, we performed terminal hemodynamic evaluation. After sedation ($100 \mu\text{g.kg}^{-1}$ and 5 mg.kg^{-1} intraperitoneal fentanyl and midazolam, respectively), animals were anesthetized by inhalation of a mixture of sevoflurane (2.5–3% sevoflurane; Penlon Sigma Delta) and oxygen, intubated for mechanical ventilation (TOPO, Kent Scientific) and placed over a heating pad (body temperature was maintained at 38°C). A peripheral venous catheter was introduced in the right femoral vein for intravenous warm Ringer's solution infusion ($8 \text{ mL.kg}^{-1}.\text{h}^{-1}$; NE-1000, New Era Pump Systems). A left thoracotomy allowed assessing the heart for insertion of a pressure-volume catheter in the LV through the apex (SPR-838 Millar Instruments, respectively). A probe (Transonics) was placed around the ascending aorta that allowed CO measurement (Active Redirection Transit Time Flowmeter, Triton Technology). Signals were continuously acquired (MPVS 300, Millar Instruments), recorded at 1000 Hz (ML880 PowerLab 16/30, ADInstruments), and analysed (PVAN 3.5, Millar Instruments). Recordings were obtained at suspended end-expiration. The LV catheter was advanced to record systemic arterial pressure. Parallel conductance was assessed with hypertonic saline. After euthanasia (100 mg.kg^{-1} intravenous pentobarbital), blood (4mL) was collected for storage (-80°C) and for volume calibration (910–1048, Millar instruments). LV volumes were varied using transient inferior vena cava constrictions by adjusting a sling around the inferior vena cava. Blood was used for volume calibration in standard wells. Heart, LV+septum, lung, skeletal muscle (gastrocnemius, soleus and tibialis anterior) were collected, weighed and immediately snap frozen on liquid nitrogen, and stored at -80°C . Tibia length was measured and then used to normalizations. Body surface area was defined as $9.1 \cdot (\text{weight})^{2/3}$ (Hamdani et al., 2013).

Biomarkers quantification

Blood samples were obtained through RV cannulation, were collected in EDTA tubes after hemodynamic evaluation. Samples were centrifuged at 5000 rpm for 15 minutes at 4°C. Serum was subsequently separated and frozen. Before the quantification, serum was centrifuged at 20 000 rpm for 15 minutes at 4°C for lipid separation. Quantitative enzyme immunoassays (ELISA) of interleukin- 6 (IL-6), IL-10, intercellular adhesion molecule-1 (ICAM-1)/CD54, metalloproteinase (MMP)-2, MMP-9, tissue inhibitor of metalloproteinase-1 (TIMP-1) (R&D Systems, #R6000B, #R1000, #RIC100, #MMP200, #RMP900, #RTM100, respectively), NT-pro-BNP, malondialdehyde (MDA), high sensitivity C-Reactive Protein (hs-CRP), TNF-alpha, vascular adhesion molecule-1 (VCAM-1) (MyBioSource, #MBS704791, #MBS727531, #MBS281245, #MBS824520, #MBS2502676 respectively) and protein carbonyl content (Abcam, #ab126287) were measured according to the manufacturer's instructions. Results were analysed using an ELISA plate reader (UVM-340, ASYS Hitech GmbH). A calibration curve was constructed by plotting the absorbance values at 450nm (with specific correction, according to manufacturer's protocol) and concentrations of unknown samples were determined.

Histological studies

Slices of LV tissue were submersed in a fixative solution of 4% paraformaldehyde by diffusion for 24 hours and subsequently dehydrated with graded ethanol and included in paraffin blocks. Xilene was used in the transition between dehydration and impregnation. LV blocks were embedded in the upright position in order to distinguish the endocardium, midwall, and the epicardium of the LV free wall in cross sections. Serial sections (5 µm of thickness) of paraffin blocks were cut by a microtome (Minot type microtome (RM2125RTS, Leica, Nussloch, Germany) and mounted on silane-coated slides. The slides were dewaxed in xylene and hydrated through graded alcohols finishing in phosphate buffered saline solution prepared by dissolving Na₂HPO₄ (1.44 g), KH₂PO₄ (0.24 g), NaCl (8 g), KCl (0.2 g) and adjusting pH to 7.2. Deparaffinized sections from LV were stained for haematoxylin-eosin, performed by immersing slides in

Mayer's haematoxylin solution for 3–4 min followed by immersion in 1% eosin solution for 7 min, dehydration with graded alcohols through xylene, and mounted with DPX. Stained sections of slides were digitally photographed (Olympus XC30 Digital colour Camera). Cardiomyocytes surface area (CSA) was measured using imaging software (cell[^]B, Olympus). We analysed 5 random animals per group, 10 photos per animal (magnification of X400) and only nuclei-centred cardiomyocytes were considered for analysis. In order to determine the amount of cardiac fibrosis, slides were immersed in picrosirius red for 2h, washed with acid water (0.05% acetic acid) and mounted after de-hydration as described before (Falcao-Pires et al., 2011b). Digital images (magnification of X400) were analysed, using image analysis software (Image-Pro Plus version 6.0, Media Cybernetics Inc.), from 5 random animals per group, 10 photos per animal.

Isolated cardiomyocyte

Force measurements were performed in single and mechanically isolated cardiomyocytes as previously described (Borbely et al., 2005; Falcao-Pires et al., 2011a). Myocardial samples (10-15 mg wet weight) from rat hearts subjected to the exercise protocol described above were defrosted in cold relaxing solution, mechanically disrupted, and incubated for 5 minutes in relaxing solution supplemented with 0.2% Triton X-100, in order to remove membrane's structures. Afterwards, single cardiomyocytes were attached between the force transducer and the piezoelectric motor with glue. Passive tension was measured at sarcomere lengths ranging between 1.8 μ m to 2.3 μ m (0.1 μ m step increases) at 15°C. Force values were normalized for myocyte cross-sectional area.

Statistical Analysis

The Shapiro-Wilk or Kolmogorov–Smirnov test was performed to check normality of the data. Between-group comparisons of normal data were performed with independent sample t-test. Non-normal data were analysed with nonparametric tests, namely the Mann-Whitney *U* Test. Data are expressed as means \pm SD. Statistical analysis was performed using the GrapPad Prism 8 and the statistical significance was set at $p < 0.05$.

RESULTS

General morphometric features

Absolute and normalized (to tibia length) morphometric parameters are presented in Table 1. In comparison to ObSED group, ObEX group presented a significant increase on gastrocnemius (1.8 vs. 2.0 mg, $p<0.001$) and tibialis anterior muscle weight (0.62 vs. 0.69 mg, $p<0.001$), even when adjusted for tibia length (43.7 vs. 52.6 mg/mm, $p<0.001$ and 16.2 vs. 18.1 mg/mm, $p=0.02$, respectively).

Table 1. General morphometric characterization.

	ObSED	ObEX
BW (g)	604 ± 49	624 ± 36
TL (mm)	38.5 ± 1.1	38.5 ± 0.8
Heart (g)	1.7 ± 0.17	1.8 ± 0.11
Heart W/TL (g.mm ⁻¹)	438 ± 42	454 ± 31
LV+IVS (g)	1.5 ± 0.18	1.6 ± 0.11
LV+IVS/TL (mg.mm ⁻¹)	40.5 ± 4.6	41.6 ± 3.2
Lung (g)	2.6 ± 0.3	2.8 ± 0.6
Lung/T (mg.mm ⁻¹)	66.7 ± 8.2	71.7 ± 16.6
Gastrocnemius (g)	1.8 ± 0.08	2.0 ± 0.10*
G/TL (mg.mm ⁻¹)	47.3 ± 1.4	52.6 ± 2.4*
Soleus (g)	0.17 ± 0.02	0.18 ± 0.02
S/TL (mg.mm ⁻¹)	4.4 ± 0.5	4.7 ± 0.7
Tibialis anterior (g)	0.62 ± 0.05	0.69 ± 0.03*
TA/TL (mg.mm ⁻¹)	16.2 ± 1.5	18.1 ± 1.1*

BW: body weight; TL: tibia length; LV+IVS: left ventricle+interventricular septum; G: gastrocnemius; S: soleus; TA: tibialis anterior. Data are mean ± SD. * $p<0.05$ vs ObSED.

Effects of exercise training on exercise tolerance

According to respiratory quotient, none of the groups reached the criteria for maximum VO_2 . The ObEX group showed increased exercise capacity compared with ObSED group (Figure 1), as shown by their greater $\text{VO}_{2\text{peak}}$

(21.1 ± 2.5 vs. 17.2 ± 1.8 mL.Kg⁻¹.min⁻¹, $p=0.005$), total running time (7.51 vs 9.22 minutes, $p=0.005$), and maximum running speed (30 vs 26 m/min, $p=0.02$).

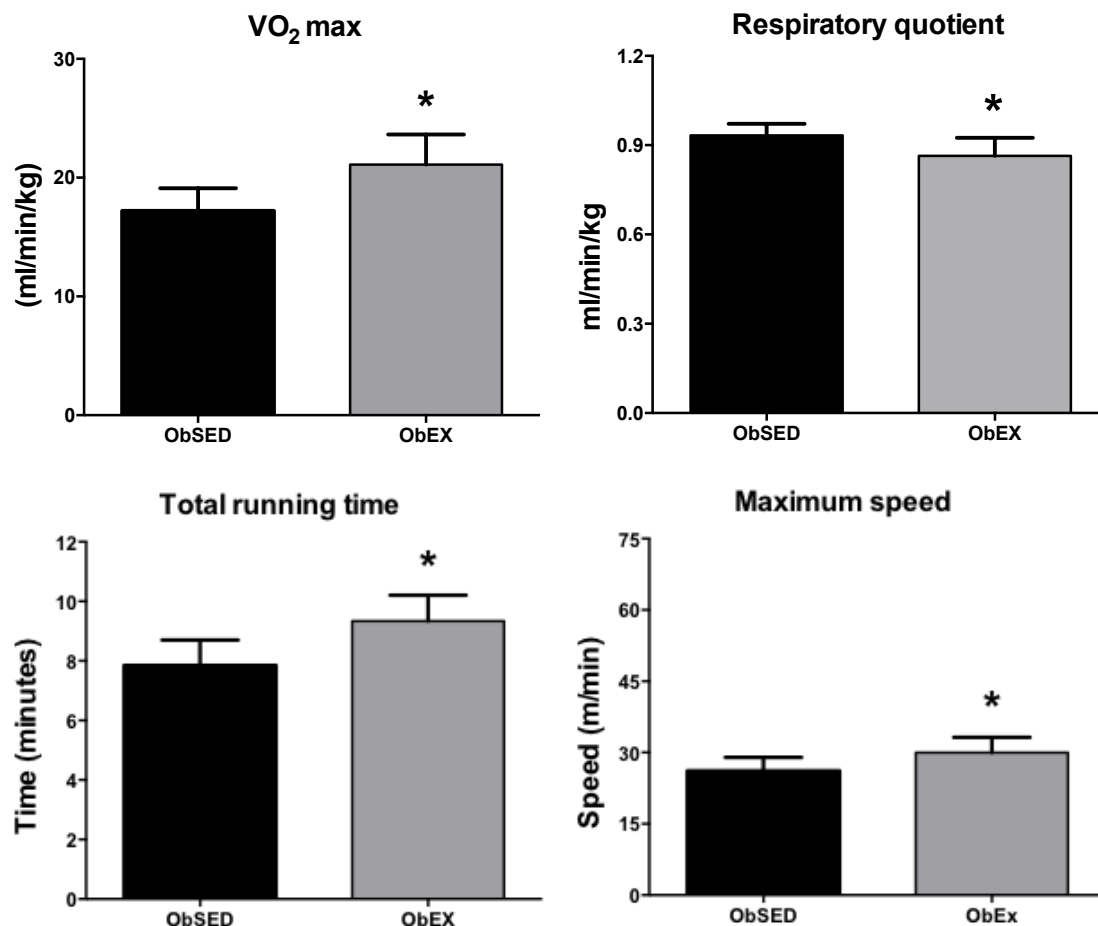


Figure 1: Exercise tolerance test data. Exercise training increased VO₂ peak, total running time and maximum running speed. VO₂ peak: peak oxygen consumption. Data are mean ± SD. * $p<0.05$ vs ObSED.

Effects of exercise training on cardiac function

Hemodynamic evaluation did not show significant differences between both groups (Table 2). However, exercise training was capable to induce a decrease in the end-diastolic pressure-volume relationship that was almost significant (0.036 vs. 0.029 $\mu\text{L.cm}^{-2}$, $p=0.07$). When compared with a reference control group, previously published by us (Hamdani et al., 2013), we found that θEDPVRi was significantly greater in the ObSED group ($p<0.05$ vs. Wistar-Kyoto), but not in

ObEX group ($p > 0.05$ vs. Wistar-Kyoto), further suggesting that the exercise training protocol improved diastolic stiffness.

Table 2. Invasive hemodynamic evaluation parameters.

	ObSED	ObEX
BSA (cm ²)	652 ± 6	660 ± 8
HR (min ⁻¹)	362 ± 9	337 ± 8
Pressure		
MAP (mmHg)	143 ± 4	132 ± 4
LVSP (mmHg)	175 ± 4	168 ± 4
LVEDP (mmHg)	11 ± 1	11 ± 1
dP/dt _{min} (mmHg.s ⁻¹)	-10662 ± 1939	-11915 ± 1926
dP/dt _{max} (mmHg.s ⁻¹)	10631 ± 2077	9789 ± 1063
τ (ms)	10.2 ± 0.5	10.3 ± 0.5
Volume		
CI (μL.min ⁻¹ .cm ⁻²)	124 ± 7	133 ± 4
EF (%)	58 ± 3	54 ± 1
LVEDVi (μL.cm ⁻²)	7.0 ± 0.7	7.6 ± 0.2
E _{ai} (mmHg. μL ⁻¹ .cm ⁻²)	392 ± 122	342 ± 30
ESPVRi (mmHg. μL ⁻¹ .cm ⁻²)	2.1 ± 0.3	2.2 ± 0.3
EDPVR β _i (μL.cm ⁻²)	0.036 ± 0.009	0.029 ± 0.003

BSA: body surface area; HR: heart rate; MAP: mean arterial pressure; LVSP: left ventricular systolic pressure; LVEDP: left ventricular end diastolic pressure; dP/dt_{min}: maximum rate of pressure fall; dP/dt_{max}: maximum rate of left ventricular pressure rise; τ : time constant of isovolumetric relaxation; CI: cardiac index; EF: ejection fraction; LVEDVi: left ventricular end diastolic volume indexed for BSA; E_{ai}: arterial elastance indexed for BSA; ESPVRi: slope of the linear end systolic pressure volume relationship with volumes indexed for BSA; EDPVRi: end diastole pressure volume relationship; β_i: chamber stiffness constant for indexed volumes, derived from exponential EDPVR. Data are mean ± SD. * $p < 0.05$ vs ObSED.

Effects of exercise training on cardiomyocyte stiffness

To evaluate whether exercise training was capable to modulate passive tension at myofibrillar level, we stretched isolated skinned cardiomyocytes at sarcomere lengths ranging from 1.8 μm to 2.3 μm (0.1 μm step increases), in order to obtain passive tension-sarcomere length relationships. Cardiomyocytes harvested from the LV free wall of ObEX presented marked downward-shifts in passive tension at sarcomere lengths relationships compared to ObSED at

myofibrillar level from 2.1 μm onward ($p < 0.05$) (Figure 2). The passive tension for the same sarcomere lengths relationships was steeper in the ObSED group, suggesting intrinsic changes at the myofibrillar level. No significant differences of active tension were observed between groups ($P > 0.05$ vs. ObSED).

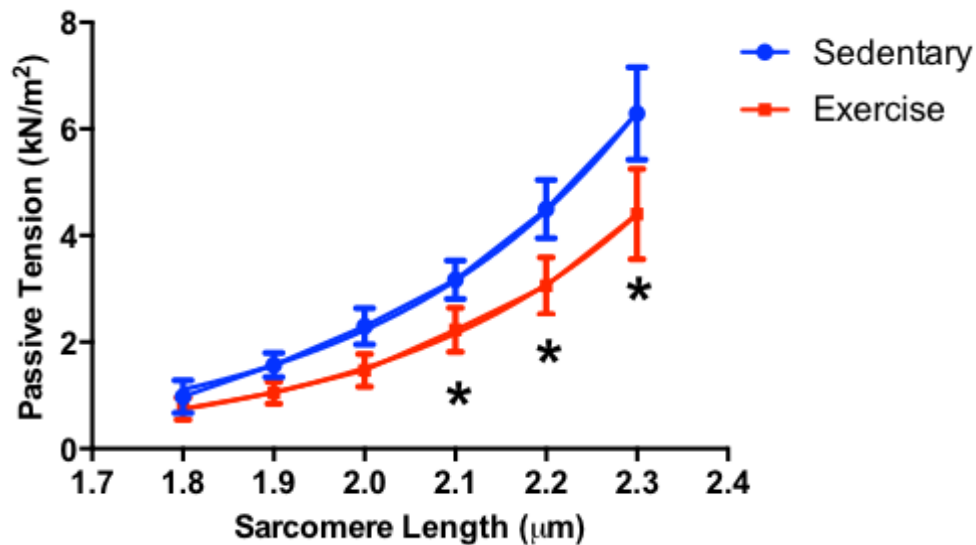


Figure 2: Effect of exercise training on passive tension in LV skinned cardiomyocytes. Exercise training result in downward-shifts in passive tension at sarcomere lengths relationships compared to ObSED at myofibrillar level from 2.1 μm onward. Data are mean \pm SD. * $p < 0.05$ vs ObSED.

Effects of exercise training on histological parameters

When analysing the cross-sectional area of LV (Figure 3A, C), no differences were detected between groups. However, the ratio collagen/muscle was significantly reduced in ObEX when compared to ObSED group (0.08 vs. 0.12 $p < 0.001$) (Figure 3D).

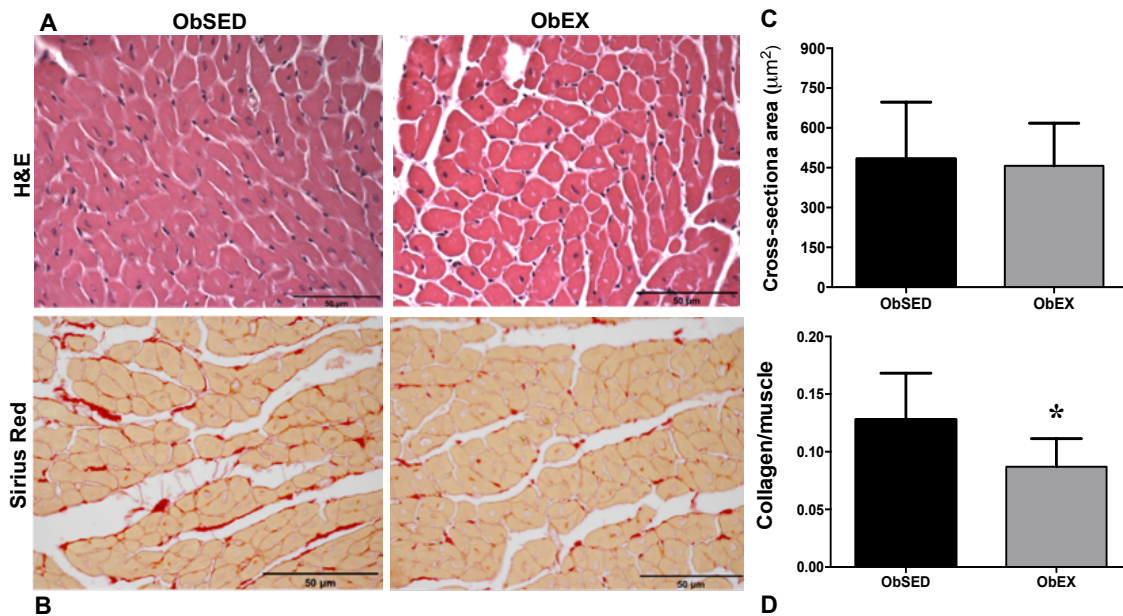


Figure 3: Effects of exercise training on histological parameters. No differences were detected in cross-section area (A-C) while exercise training reduced collagen/muscle ratio (D) in ObEX. Data are mean \pm SD. * $p < 0.05$ vs ObSED.

Effects of exercise training on plasma levels of biomarkers

Results from plasma levels of biomarkers are presented in table 3 and revealed that the expression of pro-inflammatory cytokines IL-6 ($p=0.04$), CRP ($p=0.004$) and TNF- α ($p=0.01$) were decreased in ObEX group when compared to ObSED. Similar findings were observed regarding circulating markers of oxidative stress (evaluated by PCC; $p=0.02$), endothelial activation (ICAM-1; $p=0.03$) and cardiac overload (NT-proBNP, $p=0.001$) in ObEX group. Finally, ECM remodeling markers, such as MMP-9 ($p<0.001$) and MMP-9/TIMP-1 ($p=0.03$), were also reduced in ObEX group compared with ObSED.

Table 3: Plasma levels of circulating biomarkers.

Biomarkers	ObSED (n=7)	ObEX(n=8)
IL-6 (pg/mL)	2983 ± 2084	912.4 ± 1274*
CRP (ng/mL)	7.2 ± 3.8	1.842 ± 0.8*
TNF-α (pg/mL)	509 ± 301	177±105*
ICAM-1 (pg/mL)	47492 ± 22889	28403 ± 7142*
VCAM-1 (pg/mL)	44.4 ± 51	57 ± 34
PCC (nmol/mg)	292 ± 203	151 ± 86*
MDA	19.31 ± 5	20.48 ± 8
NT-proBNP (pg/mL)	1403 ± 491.5	581.4 ± 189*
MMP-2	19728 ± 7760	19785 ± 7862
MMP-9	103 ± 29	33 ± 23*
TIMP-1	17623 ± 8778	10651 ± 4187
MMP-9/TIMP-1	0.008 ± 0.005 ^{*†}	0.003 ± 0,001*

IL-6: interleukin-6; CRP: C-reactive protein; TNF-α: tumor necrosis factor alpha; ICAM: intercellular adhesion molecule; VCAM: vascular adhesion molecule; PCC: protein carbonyl content; MDA: malondialdehyde; NT-proBNP: N-terminal pro-brain natriuretic peptide; MMP-2: matrix metalloproteinase-2; MMP-9: matrix metalloproteinase-9; tissue inhibitor of metalloproteinase-1; MMP-9/TIMP-1: matrix metalloproteinase-9/tissue inhibitor of metalloproteinase-1 ratio. Data are mean ± SD.*p<0.05 vs ObSED.

DISCUSSION

The present study demonstrates beneficial effects of moderate exercise training in the ZSF1 rat model of HFpEF. We showed that exercise training: i) improved exercise capacity, ii) attenuated LV stiffness, and iii) reduced circulating biomarkers of inflammation, endothelial activation, oxidative stress, neurohumoral activation and ECM remodeling.

Exercise intolerance is a primary symptom in patients with HFpEF, manifested by early fatigue and dyspnea, which is a strong determinant of prognosis and reduced quality of life (Upadhyaya et al., 2015). While drugs interventions have failed to improve exercise capacity and quality of life in HFpEF patients, exercise training has been shown to improve both outcomes (Fukuta et al., 2016). Using a previously validated animal model of HFpEF (Hamdani et al., 2013), the present study corroborates that exercise training improves exercise capacity in HFpEF. Although the improvement on exercise capacity by exercise training is well recognized, the physiological mechanisms underlying these changes in HFpEF are poorly understood. Current published data suggests that the greater VO_2 peak found in HFpEF patients after a training program is mainly because of non-cardiac peripheral adaptations, as no study has yet shown to improve cardiac output (Fu et al., 2016; Haykowsky et al., 2012). However, both clinical (Dieberg et al., 2015; Pearson et al., 2017) and pre-clinical studies (Hidalgo et al., 2014; Marshall et al., 2013) support the hypothesis that exercise training improves diastolic function in HFpEF setting, which may also account for a greater exercise response. Consistently, our data showed that exercise training modulates LV compliance and distensibility in HFpEF animals. We found a borderline-significant decrease in end-diastolic pressure-volume relationship, which was accompanied by reduced cardiomyocyte stiffness. In addition, exercised animals showed lower circulating levels of NT-proBNP, suggesting reduced wall stress, possibly due to a less stiffer heart (Bordbar et al., 2012). Whether exercise training can modulate LV compliance and distensibility in HFpEF remains to be explored, but these diastolic variables were shown to be modulated by exercise training in healthy middle age individuals (Howden et al., 2018) and in healthy seniors (Bhella et al., 2014).

So far, the literature does not elucidate how exercise training could improve diastolic function in HFpEF patients. Edelmann and colleagues demonstrated that exercise training improved LV diastolic function and promoted atrial reverse remodelling without change in LV mass, suggesting a switch from pathologic to physiologic hypertrophy (Edelmann et al., 2011). In addition, exercise training was associated with a significant reduction in procollagen type I plasma levels, suggesting that improvement in diastolic function may be associated with reduced collagen turnover (Edelmann et al., 2011). Our data strengthens the hypothesis that exercise training enhances cardiac function by decreasing cardiomyocyte passive tension and LV fibrosis. We observed a downward-shift in passive tension at sarcomere lengths relationships from LV cardiomyocytes of ObEX rats comparing to those extracted from ObSED. These findings confirm an increase in LV compliance promoted, at least in part, by intrinsic cardiomyocyte changes. This is supported by previous data showing that chronic voluntary running decreases titin-based passive stiffness in a phosphorylation-dependent manner in mice with diastolic dysfunction (Slater et al., 2017) (Kotter et al., 2013). In addition, it is also possible that the improved LV compliance could be due to adaptations at the ECM level, as we found that exercise training attenuated LV collagen formation and reduced systemic levels of MMP9 and MMP9/TIMP1 ratio. Similarly, treadmill exercise training was shown to attenuate diastolic impairment by reducing fibrosis in the Yucatan miniature swine model of HFpEF (aortic-banded to produce concentric LV hypertrophy) (Marshall et al., 2013). Our data suggests that the overall beneficial effect of exercise on cardiac function might be due to both cardiomyocyte's intrinsic and extrinsic mechanisms.

It has been proposed that myocardial remodelling and dysfunction in HFpEF is triggered by low-grade chronic systemic inflammation induced by comorbidities (Paulus & Tschope, 2013). This persistent inflammatory state causes coronary microvascular endothelial inflammation and oxidative damage, leading to lower nitric oxide bioavailability and PKG activity, thus contributing to LV stiffness by inducing hypertrophy and hypophosphorylation of titin (Franssen et al., 2016; Paulus & Tschope, 2013). In addition, endothelial activation of cell adhesion molecules by inflammation would favour the migration of monocytes into the

subendothelium and stimulates the conversion of fibroblasts to myofibroblasts by release transforming growth factor β (TGF- β). This would also contribute to cardiac stiffness, as myofibroblasts are responsible for depositing collagen in the interstitial space. The majority of these features have also been shown to be present in the ZSF1 obese animal model (Hamdani et al., 2013; Leite-Moreira et al., 2018). In the current work, we found that ObEX animals had reduced levels of circulating inflammatory cytokines (CRP, IL-6 and TNF- α), oxidative stress (PCC), and endothelial activation (ICAM-1). Thus, we hypothesized that these systemic effects promoted by exercise training were capable to interfere with the cascade of events leading to increased diastolic left ventricular stiffness and heart failure development.

CONCLUSION

Our findings suggest that moderate exercise training improves exercise capacity and attenuates LV diastolic stiffness in the ZSF1 obese rat model of HFpEF. This was paralleled by reduced circulating levels of inflammatory cytokines and markers of endothelial dysfunction and oxidative stress. These results provide novel mechanistic insight into the benefits of training in a clinically meaningful translational animal model of HFpEF.

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CHAPTER IV

GENERAL DISCUSSION

Methodological discussion

The rationale underlying this work is grounded in several premises and knowledge gaps. The first premise is that HFpEF continues to be refractory to available therapies, with current treatment ESC guidelines highlighting the importance of aiming to alleviate symptoms and specifically targeting patient's well-being or other health-related outcomes (Ponikowski et al., 2016). The second premise is the awareness of an urgent need for novel approaches not only for the treatment and management of HFpEF but also for its prevention by the screening and management of the potential modifiable contributing risk factors, ultimately to prevent HFpEF (Bobenko et al., 2018; Kondamudi et al., 2017). The third premise is that physical fitness or physical activity (PA) are recognized as key modifiable factors for the prevention of HFpEF and management of cardinal symptoms such as exercise intolerance and quality of life, which are the main targets currently identified by the ESC guidelines (Chan et al., 2016) (Kitzman et al., 2010; Nolte et al., 2015). From a clinical perspective, this imposes some challenges that need to be tackled to maximize the health care, which leads us to the knowledge gaps that we identified in our literature review and thus substantiate the aims of our study. **First**, it must be considered that physical fitness is a multicomponent construct and apart from cardiorespiratory fitness (CRF), we do not know how HFpEF affects the other components or which of these is better representative of health-related outcomes such as quality of life. To appraise this issue in **Study I**, we selected four tests from the physical fitness test battery for seniors proposed by Rikli and Jones (Rikli & Jones, 2013). We choose the 8-foot up and go test (8FUG) to assess dynamic balance and mobility, the 6-minute walk test (6MWT) to assess CRF, the handgrip strength test to assess upper body strength, and body mass index to assess body composition. These tests are widely used and representative of the different physical fitness components, there are reference standard values for comparison, they are easy to apply on the clinical setting and they are highly related to the daily activity demands of the senior population, which is the most representative population segment affected by HFpEF. The current approach strengthens this study once that previews studies in HFpEF patients only

evaluated one or two physical fitness components (CRF and body composition) (Edelmann et al., 2011; Kitzman et al., 2010).

Similarly to physical fitness, quality of life is also a multidimensional construct, and therefore can also be assessed according to different dimensions (e.g. general, emotional and physical) (The World Health Organization Quality of Life Assessment Group, 1998). The most frequent instruments used to assess health-related quality of life in patients with HFpEF are The 36-Item Short-Form Health Survey (SF-36) and the Minnesota Living With Heart Failure Questionnaire (MLHFQ) (Edelmann et al., 2011; Kitzman et al., 2013; Nolte et al., 2015). In **Study I**, we choose the MLHFQ because it encompasses the different dimensions of QoL (such as general, physical and the emotional) and it was specifically designed and validated to assess patient's perception of the impact of HF signs and symptoms on their life (Rector & Cohn, 1992).

A **second** identified gap is related to the methods and instruments used to assess PA, the quality and robustness of the information they provide and their usefulness in clinical context. In order to provide tailored counselling and prescription to HFpEF patients, it is crucial that the instruments to measure PA levels are accurate and reliable. In epidemiological studies encompassing large samples as well as in clinical settings, questionnaires are often used to assess and monitor PA levels. However, in healthy and unhealthy populations, PA data measured by questionnaires are not strongly associated with those obtained with objective measurement instruments, for instance those resulting from accelerometer evaluation (Lee et al., 2011). In addition, the evaluation of PA by questionnaires does not allow to obtain information on the entire spectrum of physical activities performed in the everyday life (Prince et al., 2008). This is a major flaw to the knowledge of the real PA patterns of HFpEF patients and thus limits its use for counselling and control of PA in clinical settings. Moreover, PA assessment by questionnaires are based on recall, and this is probably one of the main reasons to the observed weak-to- mild associations with the data gathered from more objective instruments. Indeed, for many subjects and specially for the advanced aged ones it is not easy to have an accurate and reliable perception of the amount and intensity of PA they performed (Cheung et

al., 2011). Beyond the aforementioned, there is no specific PA questionnaire validated to assess PA in HFpEF patients. On its turn, accelerometers provide accurate and reliable information regarding daily PA levels and the intensities at which it is performed (Gorman et al., 2014), although their use involves some financial cost and requires expertise to manage the provided data (Troiano, 2005). In order to supply the need of a reliable and valid instrument to assess PA levels in the clinical practice for HFpEF patients, we compared PA levels from IPAQ (short form) with accelerometer-derived data (Actigraph GT3X). The short form of IPAQ was selected because it was validated against accelerometer measurements in 12 countries with several populations (Craig et al., 2003), and was developed by the World Health Organization to guide policy development related to health-enhancing physical activity (<http://www.ipaq.ki.se>). Regarding the accelerometer, we opted for the Actigraph GT3X, which has been used in hundreds of large-scale studies (Martin et al., 2014; Shiroma et al., 2013), but was never used to study and assess PA in HFpEF patients. This specific accelerometer was selected because it has an activity monitor that can capture body accelerations in three different planes of motion, such as vertical, antero-posterior and medio-lateral (Zisko et al., 2015). The device provides activity counts as a composite vector magnitude (VM) of these three axes (Sasaki et al., 2011). By measuring motion in three different planes, the GT3X accelerometer has the ability of quantify PA levels more accurately than common uniaxial accelerometers (Kelly et al., 2013). While for the above-mentioned reasons we believe that PA assessed through triaxial accelerometry strengthens our study, it posed a methodological challenge as there are no cut-point validated to define PA intensities in HFpEF patients. In order to overcome that issue, we opted to select cut-points previously validated in older adults using the same device (Santos-Lozano et al., 2013), once the clinical population included in our study was mostly elderly (76 ± 6 years). Therefore, we can compare the obtained results with an aged matched reference population. However, we must recognize that these cut-points were developed in a laboratory setting during treadmill walking and not in free-living conditions (Santos-Lozano et al., 2013). This is a major issue to consider once cut-points determined in laboratory settings were

demonstrated to be less accurate to capture movements performed in free-living conditions, which may misclassify the time spent in each PA intensity (Sasaki et al., 2016). In order to overcome this limitation, we used a low frequency extension filter, which was shown to be more sensitive to capture slower and less intense body movements, such as those that frequently occur in daily life activities (Migueles et al., 2017).

Another methodological issue that deserves consideration is the sample size in **Study I** and **Study II**, which was small, and thus, limits the generalization of our results. However, we must highlight that the clinical features of our sample are very similar to the clinical features typically presented in epidemiological and clinical studies with HFpEF patients (Ponikowski et al., 2016; Vaduganathan et al., 2016). Patients with HFpEF are more likely to be women and old aged, with a higher prevalence of comorbidities such as hypertension, dyslipidaemia and obesity (Dunlay et al., 2017). Similarly, patients include in the present work were predominantly elders (76 ± 6 years old), mostly female (71%) and had a greater prevalence of comorbidities [e.g. hypertension (92%), dyslipidemia (71%) and obesity (58%)]. In addition, our patients presented reduced exercise capacity, as evidenced from the distance performed on the 6MWT (313 ± 91 meters), the elevated levels of natriuretic peptides (288.9 ± 191.5 pg/mL), and the cardiac structural alterations (left atrial volume index= 44.2 ± 11.7 mL/m²; left ventricular mass index= 231.3 ± 94.5 g/m²).

Although there is some evidence that exercise training can improve diastolic function in HFpEF patients (Chan et al., 2016), the mechanisms underlying this improvement are still unknown. The use of animal models of disease is particularly important because of the limited access to human cardiac tissue samples (Vaduganathan et al., 2016). None of the current animal models of HFpEF fully mimics the spectrum of changes found in the human phenotype such as the concomitant presence of comorbidities, cardiac functional and structural changes, and exercise intolerance (Lourenco et al., 2018). Recently, a new HFpEF model using the obese ZSF1 rat has been proposed (Hamdani et al., 2013). It is considered a robust model as these animals display hypertension, obesity, type 2 diabetes, insulin resistance, hyperinsulinemia,

hypertriglyceridemia and hypercholesterolaemia (Hamdani et al., 2013). Over time, this cardiometabolic risk model develops the main features found in humans diagnosed with HFpEF: i) reduced exercise tolerance, ii) preserved systolic function (LVEF, $LVdP/dt_{max}$, and the slope of ESPVR) and iii) diastolic dysfunction (higher E/e' , prolonged tau, elevated LVEDP, an upwards shift of LV diastolic pressure-volume relationship, and higher LV diastolic chamber stiffness constant) (Hamdani et al., 2013; Leite et al., 2015). Therefore, in **Study III**, we used the ZSF1 obese animal model of HFpEF to evaluate the effects of exercise training on LV function, structure and underlying molecular changes. Given that ZSF1 rats are obese, present exercise intolerance and impaired cardiac function, we anticipated that it would be difficult for these animals to complete an exercise training protocol. In attempt to ensure compliance, an aerobic continuous training of low-intensity protocol consisting of running on a treadmill at 20 m/min, 60 min/day, 5 days per week, during a period of 5 weeks was selected.

Discussion of the main results

The present thesis had three important findings. Data from **Study I** showed that physical fitness, particularly dynamic balance and agility, was positively correlated with all dimensions of quality of life, outperforming all the other components of physical fitness. Moreover, **Study II** demonstrated that IPAQ-SF is not an accurate instrument to assess PA levels in HFpEF patients, and that MVPA was the single PA intensity category positively associated with functional capacity and quality of life. Finally, **Study III** showed that exercise training improves exercise capacity, modulates LV stiffness and reduces circulating levels of inflammatory, oxidative stress and endothelial dysfunction markers.

Association between different components of physical fitness and dimensions of quality of life in HFpEF patients

As already stated, several studies have shown that physical fitness is an important determinant of quality of life in HFpEF (Kitzman et al., 2010; Nolte et al., 2015). Up to date, it was only established that higher levels of CRF are associated with a better quality of life, mainly the physical dimension (Edelmann et al., 2011; Kitzman et al., 2010). Our study adds novelty by showing that dynamic balance and mobility is the single physical fitness component associated with all dimensions of quality of life (general, physical and emotional). In addition, our data revealed that dynamic balance and mobility outperforms CRF in capturing HFpEF patients' quality of life. The capacity of the 8FUG test to better identify patient's quality of life might be explained by the wide range of physical factors that influence the performance in this test (lower body strength, balance, walking, agility and gait speed) (Rikli & Jones, 2013), which are also analogous to the required in the normal daily tasks of an independent and autonomous life (Mlinac & Feng, 2016) and perceived as the most important determinants of patient's quality of life. Although CRF assessed by maximal or symptom-limited graded exercise tests or by the six-minute walk test were proven to be associated with prognostic indicators and able to discriminate the grade of severity of disease course, it might be possible that the continuous nature of the exercise might not be sensitive to show an association with the dimensions of perceived quality of

life that is probably more influenced by the intermittent physical demands related of household activities and commuting. In this sense, results from **Study I** gave some insights from a new instrument to be used in the clinical setting, since the underlying capacities are important to monitor HFpEF patients' quality of life correlates. Despite that, the 8FUG test has the advantage of being simple to administer, requiring a limited space, minimal equipment (i.e. markers on the floor and a chair) and it is easily understood by patients (Wilkinson et al., 2018). Therefore, we recommend the use of the 8FUG test on clinical practice in combination with the assessment of patients' quality of life.

The utility of IPAQ short form to measure PA levels in HFpEF patients

Despite the evidence shows that questionnaires are valid and reliable in measuring PA, their correlation with accelerometers-derived data is far from satisfactory (Koolhaas et al., 2018; Lee et al., 2011; Prince et al., 2008; Skender et al., 2016), which limits PA-based decisions in the clinical setting regarding counselling and recommendations. Our results corroborate previous findings in the general population, showing that the measures derived from IPAQ-SF underestimates sedentary time and overestimates time spent in MVPA in HFpEF patients, and also showed poor agreement between self-reported and accelerometer-derived PA measures. Therefore, based on our findings, the use of IPAQ-SF should not be recommended to support accurate recommendations in this specific population. For clinical purposes, at an individual level, it is important to have accurate and reliable PA information to assess the risk level and to prescribe life-style changes. Therefore, the use of accelerometers to assess daily PA levels in HFpEF patients seems to have a particular importance. Objective measures can avoid bias related with subjective measures, especially in populations that have limited physical function and no experience performing PA. Moreover, self-reported measures depend on recall which can be biased specially in older patients (Prince et al., 2008). Nevertheless, although accelerometry is considered a better methodology to assess PA when compared to questionnaires, it cannot provide comprehensive information in terms of type and context in which PA occur. In this sense, to provide tailored

recommendations in the clinical setting, we suggest the use of accelerometers together with a diary log to better characterize patients' PA levels.

Patterns of PA and sedentary time in HFpEF patients, and its association with prognostic indicators

Given the growing recognition of the impact of PA levels in the prognosis of HFpEF patients, a rigorous characterization of their patterns is crucial for prescribing tailored life-style changes to improve well-being and quality of life, and to mitigate/attenuate the disease progression. In the study II we observed that HFpEF patients spent most of their waking time in sedentary behaviours while their daily activity was mainly comprised of light physical activity, and just a few minutes in MVPA. Increased sedentary behaviours and decreased time spent in MVPA are both associated with worse clinical outcomes in HFpEF patients (Hegde et al., 2017; Snipelisky et al., 2017). Interestingly, our study adds the novelty that only time spent in MVPA was significantly associated with important clinical outcomes as VO_2 , 6MWT performance and QoL, but not with E/e' or BNP levels. Thus, it seems that intensity is a requirement for significantly impacting the patient's prognosis. Corroborating this hypothesis, it was recently shown a dose-response relationship between MVPA and risk of hospitalization or mortality in HFpEF patients (Hegde et al., 2017). Despite that, it was already shown that any increase in the amount of PA may translate into some health benefits (Physical Activity Guidelines Advisory Committee Scientific Report, 2018). Taking together, these data emphasise the clinical importance to educate patients about the importance of engaging in more MVPA throughout the day and to reduce their time spent in sedentary behaviours in order to achieve higher health-related benefits.

Effects of exercise training in an animal model of HFpEF

Exercise training is emerging as an important ally for the clinical management of health-related outcomes in HFpEF patients. However, the literature thus far cannot elucidate the mechanisms by which exercise training can induce improvements in HFpEF patients. Current published data suggests

that the improvements in VO_2peak found in HFpEF patients after an exercise training program are mainly due to non-cardiac peripheral adaptations, as no study has yet shown to improve peak cardiac output (Fu et al., 2016; Haykowsky et al., 2012). However, both clinical (Dieberg et al., 2015; Pearson et al., 2017) and pre-clinical studies (Hidalgo et al., 2014; Marshall et al., 2013) support the hypothesis that exercise training improves diastolic function in HFpEF setting, which may also account for an improved acute exercise response. Results from **Study III** show that exercise training can improve exercise capacity and enhances cardiac function by decreasing cardiomyocyte passive tension and by attenuating LV fibrosis in HFpEF animals. These results provide novel mechanistic insights into the benefits of regular exercise training in a clinically translational animal model of HFpEF. Understanding the pathways that mediate these benefits, and learning how to manipulate them *in vivo*, could yield novel therapeutic approaches based on agents that mimic or potentiate the physiological benefits of exercise. As mentioned in the first chapter (General Introduction, page 9) low-grade chronic systemic inflammation derived from comorbidities is responsible for coronary microvascular inflammation, which might lead to myocardial dysfunction and remodelling. Our results in Study III suggest that beyond the improvements in cardiomyocyte passive tension and LV fibrosis, regular exercise training was able to attenuate systemic inflammation and to diminish circulating markers of oxidative stress and endothelial dysfunction. Collectively, these results empower the hypothesizes that low-grade chronic systemic inflammation is the main trigger of HFpEF. Therefore, our results confirm the pleiotropic effects of exercise training, namely its anti-inflammatory effect, which might modulate improvements in cardiac function by interfering in the cascade of events that lead to decreased diastolic left ventricular stiffness and interstitial fibrosis. Overall, results from **Study III** suggest that exercise training may be a particularly powerful adjuvant treatment option to HFpEF patients, but clinical studies are needed to clarify the best type of training, frequency and intensity in large populations with HFpEF.

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CHAPTER V

CONCLUSIONS AND FUTURE DIRECTION

CONCLUSIONS

Based on the general conclusions of each study that were presented in this thesis, it is possible to outline the following major conclusions:

- Physical fitness, particularly dynamic balance and mobility, is associated with all dimensions of quality of life in patients with HFpEF. In addition, dynamic balance and mobility outperforms others physical fitness components in capturing HFpEF patients' quality of life.
- The IPAQ-SF underestimates sedentary time and overestimates time spent in MVPA in patients with HFpEF. Patients spent only a minority of their daily time involved in MVPA, which was the only PA intensity positively associated with functional capacity and quality of life.
- Moderate exercise training improves exercise capacity and attenuates left ventricle diastolic stiffness in the ZSF1 obese animal model of HFpEF. This was paralleled by reduced circulating levels of inflammatory cytokines and markers of endothelial dysfunction and oxidative stress.

FUTURE DIRECTIONS

Further prospective cohort studies with a larger sample size are needed to strengthen or refute our conclusions that dynamic balance and mobility is more discriminative to capture HFpEF patients' quality of life, and to verify the agreement between PA measurements from the IPAQ-SF and PA measures derived from triaxial accelerometer.

In addition, experimental studies exploring the molecular changes underlying the cardiomyocyte's intrinsic and extrinsic changes induced by exercise training are fundamental as they may lead to the identification of therapeutic molecular targets.

Moreover, different exercise training protocols with the ZSF1 animal model are also required in order to better understanding the dose-response relationship between exercise training and HFpEF improvements.

APPENDICES

As seguintes questões procuram avaliar em que medida a sua insuficiência cardíaca (problema cardíaco) afetou a sua vida no último mês (4 semanas). Em cada questão, assinale com um círculo o número que melhor reflete o modo como a sua vida foi afetada (0, 1, 2, 3, 4 ou 5). Se alguma questão não se aplicar a si, assinale o número 0 (zero).

No último mês (4 semanas), a sua insuficiência cardíaca impediu-o(a) de viver como queria porque...	Não	Muito Pouco					Muito
1. lhe provocou inchaço nos tornozelos ou pernas?	0	1	2	3	4	5	
2. o(a) obrigou a sentar-se ou deitar-se para descansar durante o dia?	0	1	2	3	4	5	
3. lhe causou dificuldade em caminhar ou subir escadas?	0	1	2	3	4	5	
4. lhe dificultou a realização das tarefas domésticas ou no quintal?	0	1	2	3	4	5	
5. dificultou as suas saídas de casa?	0	1	2	3	4	5	
6. o impediu de dormir bem de noite?	0	1	2	3	4	5	
7. dificultou o seu relacionamento e as atividades com amigos ou familiares?	0	1	2	3	4	5	
8. dificultou a realização das suas atividades profissionais?	0	1	2	3	4	5	
9. dificultou as suas atividades de lazer, desportivas ou os seus passatempos?	0	1	2	3	4	5	
10. dificultou a sua atividade sexual?	0	1	2	3	4	5	
11. o(a) fez comer menos quantidade das comidas de que gosta?	0	1	2	3	4	5	
12. lhe causou falta de ar?	0	1	2	3	4	5	
13. o(a) fez sentir-se cansado(a), fatigado(a) ou com pouca energia?	0	1	2	3	4	5	
14. o(a) obrigou a internamento hospitalar?	0	1	2	3	4	5	
15. lhe criou despesas com cuidados médicos?	0	1	2	3	4	5	
16. fê-lo(a) sentir efeitos secundários provocados pela medicação?	0	1	2	3	4	5	
17. fê-lo(a) sentir-se um fardo para a família e amigos?	0	1	2	3	4	5	
18. fê-lo(a) sentir perda de autocontrolo na sua vida?	0	1	2	3	4	5	
19. fê-lo(a) sentir-se preocupado(a)?	0	1	2	3	4	5	
20. lhe dificultou a concentração ou a memória?	0	1	2	3	4	5	
21. fê-lo(a) sentir-se deprimido(a)	0	1	2	3	4	5	

Atividade Física

Estamos interessados em conhecer os diferentes tipos de atividade física, que as pessoas fazem no seu cotidiano.

As questões que lhe vou colocar, referem-se à semana imediatamente anterior, considerando o tempo em que esteve fisicamente ativo/a. Por favor, responda a todas as questões, mesmo que não se considere uma pessoa fisicamente ativa. Vou colocar-lhe questões sobre as atividades desenvolvidas na sua atividade profissional e nas suas deslocações, sobre as atividades referentes aos trabalhos domésticos e às atividades que efetuou no seu tempo livre para recreação ou prática de exercício físico / desporto.

Ao responder às seguintes questões considere o seguinte:

Atividades físicas vigorosas referem-se a atividades que requerem um esforço físico intenso que fazem ficar com a respiração ofegante.

Atividades físicas moderadas referem-se a atividades que requerem esforço físico moderado e tornam a respiração um pouco mais forte que o normal.

Ao responder às questões considere apenas as atividades físicas que realize durante pelo menos 10 minutos seguidos

Q.1 Diga-me por favor, nos últimos 7 dias, em quantos dias fez actividades físicas vigorosas, como por exemplo, levantar objetos pesados, cavar, ginástica aeróbica, nadar, jogar futebol, andar de bicicleta a um ritmo rápido?

Dias

Q.2 Nos dias em que pratica actividades físicas vigorosas, quanto tempo em média dedica normalmente a essas actividades?

Horas Minutos (por dia)

Q.3 Diga-me por favor, nos últimos 7 dias, em quantos dias fez actividades físicas moderadas como por exemplo, carregar objectos leves, caçar, trabalhos de carpintaria, andar de bicicleta a um ritmo normal ou ténis de pares? Por favor não inclua o “andar”.

Dias

Q.4 Nos dias em que faz actividades físicas moderadas, quanto tempo em média dedica normalmente a essas actividades?

Horas Minutos (por dia)

Q.5 Diga-me por favor, nos últimos 7 dias, em quantos dias andou pelo menos 10 minutos seguidos?

Dias

Q.6 Quanto tempo no total, despendeu num desses dias, a andar/caminhar?

Horas Minutos (por dia)

Q.7 Diga-me por favor, num dia normal quanto tempo passa sentado? Isto pode incluir o tempo que passa a uma secretária, a visitar amigos, a ler, a estudar ou a ver televisão.

Horas Minutos (por dia)

